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UNIVERSITY OF ALBERTA

CYANOBACTERIAL TOXIN OCCURRENCE IN SOME PRAIRIE DRINKING WATER SOURCES

BY

SANDRA KENEFICK

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE

IN

ENVIRONMENTAL SCIENCE
DEPARTMENT OF CIVIL ENGINEERING

EDMONTON, ALBERTA
SPRING, 1991

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Cyanobacterial Toxin Occurrence in Some Prairie Drinking Water Sources** submitted by **Sandra L. Kenefick** in partial fulfillment of the requirements for the degree of **Master of Science in Environmental Science**.

Dr. S.E. Hrudey (Supervisor)

Dr. P.M. Huck

Dr. G.R. Finch

E E Prepas

Dr. E.E. Prepas

Date: April 14, 1991

ABSTRACT

A number of lakes and agricultural dugouts in Alberta were surveyed to establish the prevalence of toxin producing cyanobacteria. Water bodies used as raw water supplies were selected for study because of their immediate relevance to human health. This study evaluated the relation between toxins and odorous compounds known to be produced by the cyanobacteria. Toxin release to the water phase from cyanobacterial cells, following treatment with copper sulfate and lime, was studied to evaluate the impact of algal control strategies. Hepatotoxin concentrations were analysed by high-performance liquid chromatography while neurotoxin concentrations were analysed by gas chromatography/mass spectrometry.

The hepatotoxin microcystin LR was detected in three lakes which serve as raw water sources for municipal water treatment plants. The neurotoxin anatoxin-a was not detected in these lakes. Neither microcystin LR, microcystin RR, nor anatoxin-a were detected in any of the agricultural dugouts surveyed.

Limited monitoring of the raw and treated water samples showed no detectable microcystin LR, except for one raw water sample at the detection limit. However, calculations to evaluate possible exposure scenarios suggest that estimated safe drinking water levels could be exceeded for the type of circumstances observed. No relationship was found between odour-causing agents and toxin production, indicating that odour was not a reliable indicator of cyanobacterial toxin presence.

Treatment of cyanobacterial blooms with copper sulfate was found to cause a rapid and potentially dangerous release of hepatotoxins to the water phase, but treatment with lime did not cause the same hazardous release of toxin. These findings are of importance in the management of drinking water sources which are vulnerable to cyanobacterial blooms.

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LIST OF ABBREVIATIONS

A	-L-alanine
Aba	-amino isobutyric acid
Adda	-3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl-deca-4,6-dienoic acid
ALT	-alanine aminotransferase
AST	-aspartate aminotransferase
D-Ala	-D-alanine
D-Glu	-D-glutamic acid
DOC	-dissolved organic carbon
GC/MS	-gas chromatography using mass spectrometry as the method of detection
F	-L-phenylalanine
GAC	-granular activated carbon
HPLC	-high-performance liquid chromatography
IC	-inorganic carbon
in vitro	-in laboratory cultures outside the organism
in vivo	-within the organism
I.P.	-intra-peritoneal: within the peritoneal cavity of the abdomen
isoAsp	-β-methylaspartic acid
L	-L-leucine
LDH	-lactate dehydrogenase
M	-L-methionine
Masp	-erythro-β-methylaspartic acid
Mdha	-N-methyldehydroalanine
2-MIB	-2-methylisoborneol

PAC	-powdered activated carbon
R	-L-arginine
SEM	-scanning electron microscopy
TC	-total carbon
TEM	-transmission electron microscopy
TOC	-total organic carbon
Y	-L-tyrosine

1. INTRODUCTION

This research was part of a preliminary study of the biogenic compounds produced by the freshwater cyanobacteria found in some lakes and reservoirs in Alberta. Water bodies used as drinking water supplies were selected for this project because of their immediate relevance to human health. Cyanobacteria is a scientific name for microorganisms which are commonly referred to as blue-green algae. There is an ongoing controversy among taxonomists as to whether they should be classified as bacteria or algae (Fay 1983). Although they are photosynthetic and contain photosynthetic pigments like algae, they do not have a defined nucleus, and are therefore classified as bacteria. Cyanobacteria are often referred to as blue-green algae in the literature and both terms will be used interchangeably in this document.

A number of cyanobacterial species are found in temperate inland waters with high concentrations of phosphorus and nitrogen. In addition, some cyanobacteria are able to fix atmospheric nitrogen and can dominate when inorganic nitrogen reserves have been depleted, as long as adequate phosphorus is present. A general trend toward an increase in nutrient concentrations in lakes due to anthropogenic eutrophication is likely continuing on the prairies (Carmichael 1988; Codd et al. 1989). Thus, an increasing prevalence of blue-green algae appears to be inevitable

1.1 Background

Cyanobacteria occur naturally throughout the year in many prairie lakes, but during warm, calm summer months the algal populations grow very rapidly and algal "blooms" can be seen. The water will appear green near the surface and a bluish green scum will wash up on the shore. In addition to being aesthetically displeasing, some cyanobacteria species produce hepatotoxic compounds which cause acute liver damage if the algae are ingested, while ingestion of species that produce neurotoxic compounds leads to a disruption of neuron transmission in the central nervous system.

Inadvertent consumption of poisonous algae by domestic livestock or pets is not always reported because it is not often understood that algal poisoning is a possible cause of animal deaths. Reported incidents of livestock poisonings caused by blue-green algal ingestion date back to the late 19th century (Francis 1878). Cyanobacteria have also been suspected in a number of cases of human illness over the last century (Schwimmer and Schwimmer 1968). Incidents have been documented in South Africa, Great Britain, Finland, Australia, and the United States, but research into the extent of problems in Canada has been minimal. Pioneering work by Dr. Paul Gorham and his student Dr. Wayne Carmichael made a major contribution to the understanding of toxic cyanobacteria in Canadian lakes.

The first Canadian report of livestock deaths due to cyanobacterial poisoning was from Alberta during the summer of 1917. Animal deaths were reported near Fort Saskatchewan, Lougheed and Lake Saskatoon (Schwimmer and Schwimmer 1968). There have been numerous algal poisonings reported in Canada since then, and most incidents occurred in prairie lakes or dugouts.

1.2 Scope of Project

This project involved the characterization of raw water samples collected from prairie lakes which are currently used as municipal drinking water supplies. A number of agricultural dugouts, which are used as water supplies by livestock and/or rural residents, were also sampled. The primary goal of the study was to identify the cyanobacteria present in some Alberta lakes and dugouts during the summer of 1990 and to analyse samples for some of the biogenic products of these algae. This included known blue-green algal toxins, as well as a number of known odour causing agents, which are thought to be indicators of some cyanobacterial species.

Three central Alberta lakes were chosen for the survey. All three of these lakes serve as raw water supplies for communities which utilize conventional water treatment, prior to distribution. The water treatment plant that draws its raw water from Coal Lake (Figure 1.1) serves the City of Wetaskiwin, which has a population of just over 10,000 (Alberta Bureau of Statistics, 1990). Coal Lake has an estimated volume of 38.8 x 10^6 m^3 and is approximately 10.9 km^2 in area, with an average depth of 3.5 m (Mitchell and Prepas 1990). Driedmeat Lake (Figure 1.1) is the raw water source for the water treatment plant which serves Camrose, a city with a population of nearly 13,000 (Alberta Bureau of Statistics, 1990). Driedmeat Lake has an estimated volume of 41.9 x 10^6 m^3 and is approximately 16.5 km^2 in area, with an average depth of 2.2 m (Mitchell and Prepas 1990). The water treatment plant for the village of Ferintosh serves only approximately 125 people. The village draws its raw water from Little Beaver Lake which is approximately 0.75 km^2 in area, with an average depth of 2 m (Jackson 1991).

The agricultural dugouts which were sampled for this study are located throughout north-central Alberta (Figure 1.2). There were two dugouts sampled at the Helbig sight, one at the Pretzlaff location and five in the Peace River region. Each of the dugouts chosen for this study had detailed baseline limnological data and a limited history of past algicide treatments.

The project also involved a study of the effect of treatment of algal cells with calcium hydroxide and with copper sulfate, two chemicals used in the treatment of algal blooms. This secondary study focussed on the release of algal toxins to the water phase after treatment with the respective algicides.

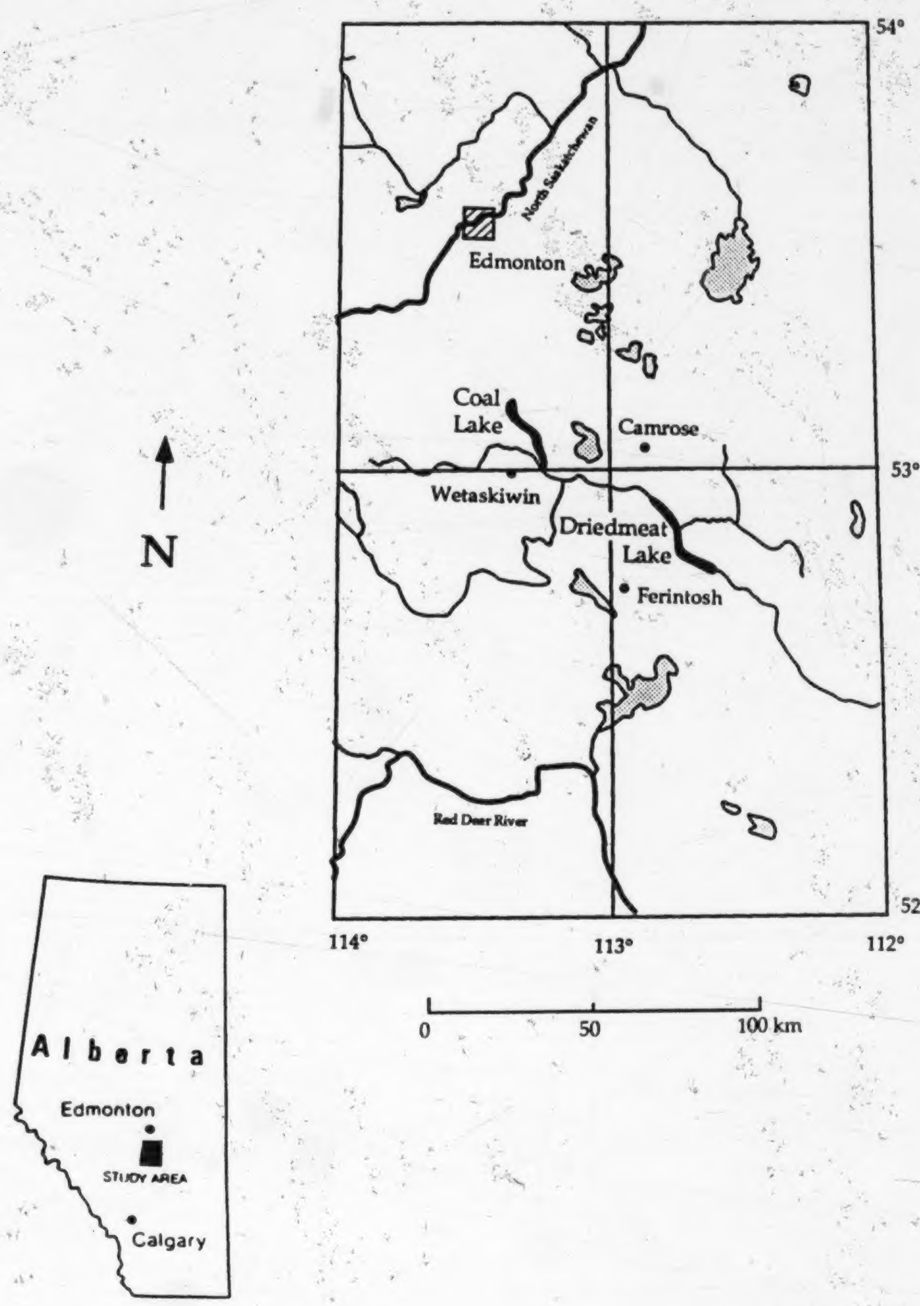

54°
North Saskatchewan
Edmonton
Coal Lake
Camrose
53°
Wetaskiwin
Driedmeat Lake
Ferintosh
Red Deer River
52°
114°
113°
112°
0
50
100 km
N
Alberta
Edmonton
STUDY AREA
Calgary

Figure 1.1 Lakes Studied

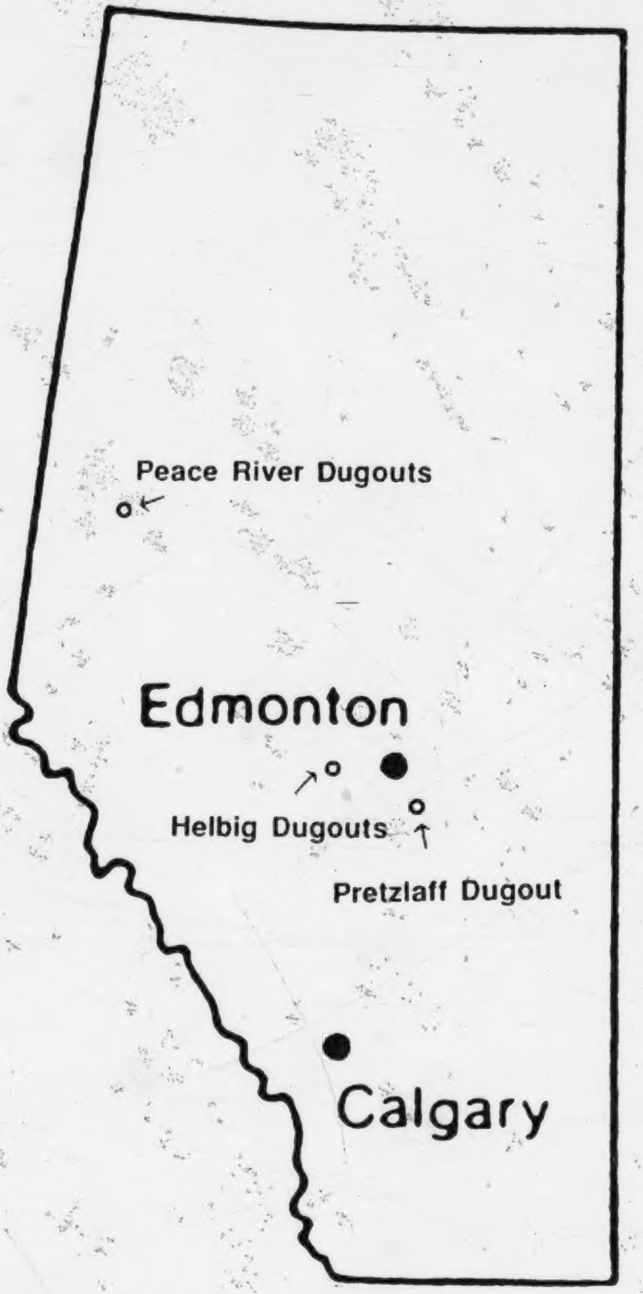

Peace River Dugouts
Edmonton
Helbig Dugouts
Pretzlaff Dugout
Calgary

Figure 1.2 Agricultural Dugouts Studied

2. REVIEW OF LITERATURE

There is a widespread misconception that naturally produced compounds are not as hazardous as those which are synthetic. Public and professional concern has emphasized the study of the environmental properties and toxicity of many synthetic compounds, but the research into natural product toxicities has received much less public and regulatory attention. Fortunately there has been substantial research concerning the toxins produced by various species of cyanobacteria.

2.1 Cyanobacterial Toxins

The first written report of an animal death due to blue-green algal toxin ingestion was by Francis in 1878. World-wide, there have been a significant number of reported animal deaths due to ingestion of water containing blue-green algal cells (Gentile 1970, Carmichael and Mahmood 1984, Carmichael and Schwartz 1984). Studies of such toxic events in Canadian waters are limited, although toxin producing cyanobacterial species are found throughout Canadian prairie lakes (Schwimmer and Schwimmer 1968).

2.1.1 Toxin Producing Cyanobacterial Species

There are a large number of cyanobacterial species, but not all of these species have been reported to produce toxins. The genera of the cyanobacteria which have been reported as toxin producers include, *Anabaena* (Gorham 1964), *Microcystis* (Botes et al. 1984), *Aphanizomenon* (Sasner 1981), *Oscillatoria* (Østensvik et al. 1981), *Nodularia* (Francis 1878), *Lyngbya* (Moore 1981), *Synechocysitis* (Carmichael 1982) and *Coelospharium* (Gorham 1964). Documented outbreaks of algal poisonings in Canadian prairie lakes have generally been limited to the *Microcystis*, *Anabaena*, and *Aphanizomenon* genera. The three species most commonly found in Alberta lakes include: *Microcystis aeruginosa*, which produces at least six hepatotoxins, *Anabaena flos-aquae*, which produces two hepatotoxins and two neurotoxins, and *Aphanizomenon flos-aquae*, which produces two neurotoxins. The toxins which can be produced by these species are listed in Table 2.1.

2.1.2 Chemical Structure of Freshwater Toxins Identified

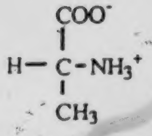
The majority of cyanobacterial toxin research has concentrated on the hepatotoxins produced by *Microcystis aeruginosa* and *Anabaena flos-aquae*. Most of this work has been carried out over the last thirty years. The first reference to the toxin produced by *M. aeruginosa* was in the late 1950's when the compound responsible for what was referred to as the fast death factor (FDF) was isolated from a laboratory culture of *M. aeruginosa* (Bishop et al. 1959). Botes et al. (1982) and Santikarn et al. (1983) were the first to determine some specific structural details of the hepatotoxins, with Botes et al. (1985) concluding that they are cyclic heptapeptides. These peptides have molecular weights of approximately 1000 and have five common amino acids (Figure 2.1) which include three D-amino acids; alanine (D-Ala), erythro-β-methylaspartic acid (Masp), and glutamic acid(D-Glu), as well as N-methyldehydroalanine (Mdha) and a nonpolar side chain of 20 carbons (3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl-deca-4,6-dienoic acid) (Adda). Each peptide also has two L-amino acids which vary with each toxin. The most common toxins have L-amino acid combinations of tyrosine, arginine, methionine, leucine or alanine (Figure 2.2). The documented combinations

Table 2.1 Reported Toxins of Freshwater Cyanobacteria

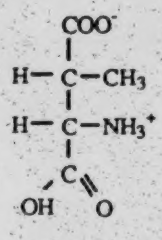
Organism	Toxin Term Used	Structural Class
Microcystis aeruginosa		
	microcystin LR[1]	peptide
	microcystin YR[1]	peptide
	microcystin LA[1]	peptide
	microcystin YA[1]	peptide
	microcystin YM[1]	peptide
	microcystin RR[2]	peptide
	microcystin FR[3]	peptide
	microcystin Laba[3]	peptide
	microcystin LR(isoAsp)[1]	peptide
Aphanizomenon flos-aquae		
	saxitoxin[1] (aphantoxin II)	alkaloid
	neosaxitoxin[1] (aphantoxin I)	alkaloid
Anabaena flos-aquae		
	anatoxin-a[1]	alkaloid
	anatoxin-a(s)[1]	unknown
	microcystin LR[1]	peptide
	microcystin LR(isoAsp)[1]	peptide

(the above information was adapted from the following sources:)

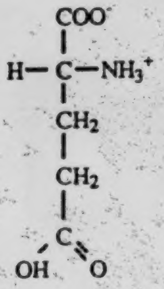
1. Carmichael 1988
2. Harada et al. 1988
3. Gathercole and Thiel 1987



alanine

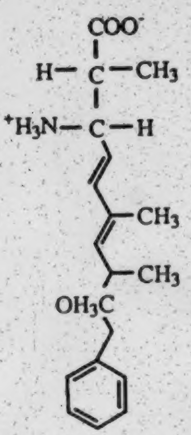


erythro-ß-methylaspartic acid

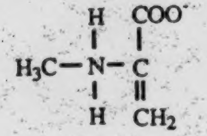


glutamic acid

D-amino Acids



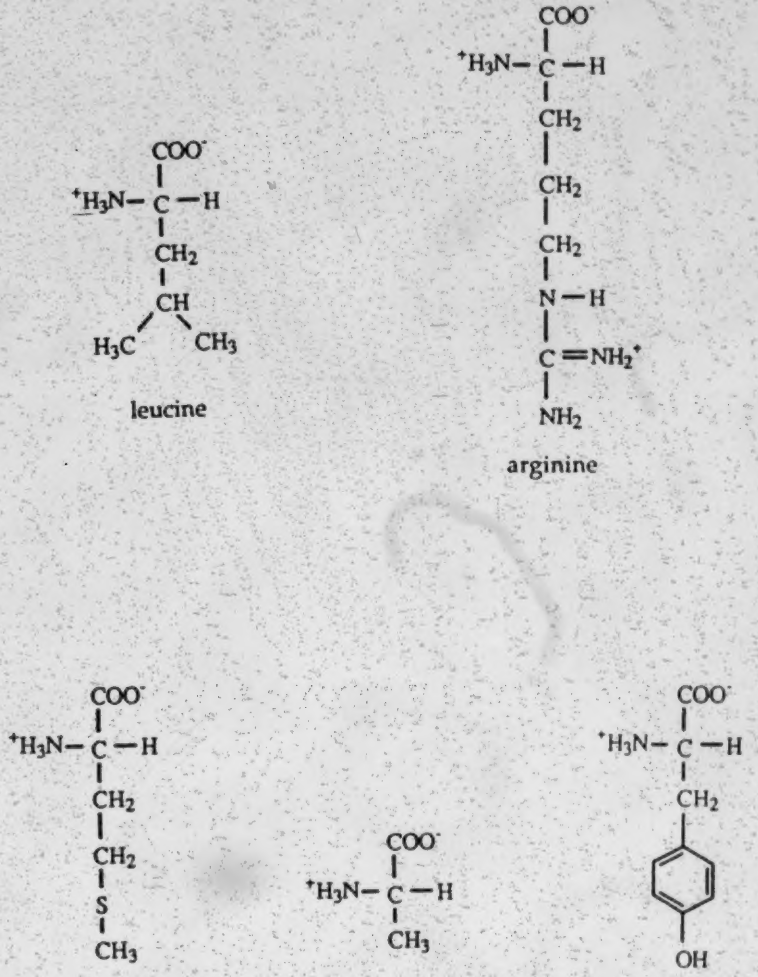
3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl-deca-4,6-dienoic acid



N-methyldehydroalanine

Unique Amino Acids

Figure 2.1 Chemical Structures of Amino Acids Common to Microcystin Toxin Structures



leucine

arginine

methionine

alanine

tyrosine

Figure 2.2 Chemical Structures of Varying Amino Acids in Microcystin Toxin Structures

include: leucine-alanine (microcystin LA), leucine-arginine (microcystin LR), tyrosine-arginine (microcystin YR), tyrosine-alanine (microcystin YA) and tyrosine-methionine (microcystin YM). The basic microcystin structure is:

—D-Ala---I---Masp—II---Adda---D-Glu---Mdha—

where I and II are the variable L-amino acids. Figure 2.3 illustrates the complete structure of microcystin LR. Another hepatotoxin produced by both *Microcystis aeruginosa* and *Anabaena flos-aquae* has the leucine-arginine combination but has aspartic acid instead of β-methylaspartic acid (microcystin LR(isoAsp)) (Carmichael 1988). Work by Gathercole and Theil (1987) suggested two further combinations; microcystin FR (phenylalanine and arginine) and microcystin LAba (leucine and amino isobutyric acid). Another hepatotoxin isolated by Harada et al. (1988), contained two arginine L-amino acids (microcystin RR).

There have been two groups of neurotoxins isolated from freshwater cyanobacteria. These include the anatoxins which are produced by *Anabaena flos-aquae*, and the saxitoxins produced by *Apanizomenon flos-aquae*. Six toxins were originally thought to have been isolated from cultures of the *Anabaena* species (Carmichael and Gorham 1978), although only two toxins have been studied in detail since then.

Anatoxin-a is an alkaloid produced by *Anabaena flos-aquae* and was the first anatoxin to be chemically defined. It is a potent depolarizing neuromuscular blocking agent (Devlin et al. 1977; Carmichael et al. 1979).The IUPAC name for anatoxin-a is 1-9-azabicyclo [4.2.1] non-2-en-2-yl-ethanone and the structure is shown in Figure 2.4.

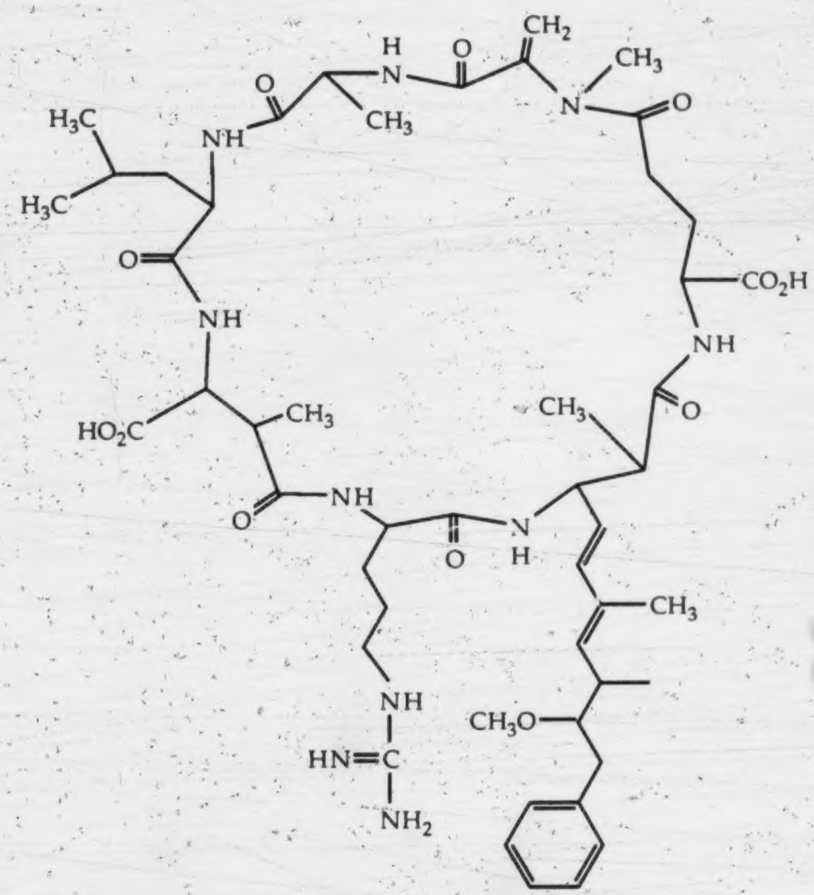
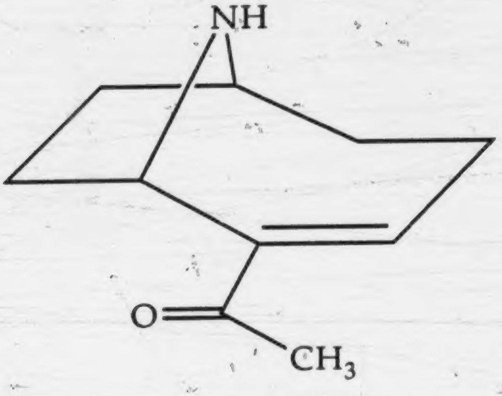
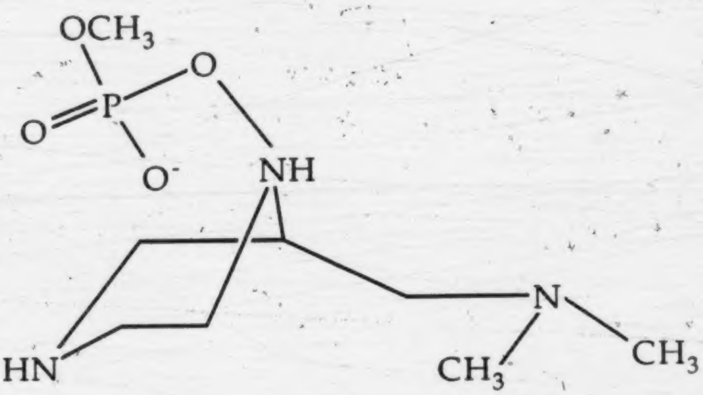

H
O
CH2
N
CH3
O
H3C
NH
CH3
N
O
H3C
O
CO2H
NH
NH
CH3
CH3
O
HO2C
NH
O
N
O
H
CH3
NH
CH3O
HN=C
NH2

Figure 2.3 Chemical Structure of Microcystin LR



Structure of anatoxin-a


OCH3
O
P
O
O-
NH
HN
N
CH3
CH3

Structure of anatoxin-a(s)

Figure 2.4 Chemical Structures of Anatoxins

The second well documented *Anabaena* toxin is anatoxin-a(s), which was given the additional "(s)" designation because test animals salivated when exposed to the toxin (Carmichael and Gorham 1978). Matsunaga et al. (1989) reported that anatoxin-a(s) is a unique phosphate ester of a cyclic N-hydroxyguanidine (Figure 2.4). The anatoxin-a(s) toxicity is attributed to exceptional anticholinesterase activity (Mahmood and Carmichael 1986b).

The neurotoxins which are produced by *Aphanizomenon flos-aquae* have been shown to behave chemically and physically similar to the well documented paralytic shellfish poisons, saxitoxin and neosaxitoxin but the structures are not fully documented (Mahmood and Carmichael 1986a).

2.1.3 Toxic Effects

Documented cases of animal deaths due to cyanobacterial toxins date back to the late 1800's and the documented cases of adverse effects on human health caused by cyanobacterial toxins go back more than 60 years. Researchers have studied the acute effects of the toxins in detail, but the chronic effect of the toxins has received relatively limited attention. Acute effects are the primary concern when studying neurotoxins, but in the case of hepatotoxins, acute effects as well as chronic exposure effects must be considered.

There are apparently no documented and reported cases of human death due to cyanobacterial poisoning (Gorham and Carmichael 1988), although experiences in many parts of the developing world may not be fully documented. However, humans do not intentionally ingest the algal biomass which will normally contain the largest portion of any toxin present. Likewise, humans will generally be reluctant to consume water from a source which is experiencing a heavy bloom. There have been a number of cases of human illness due to unintentional ingestion of small amounts of water contaminated with toxic algal cells or continuous ingestion of water with very low levels of dissolved algal toxins. Detailed descriptions of cases of human gastroenteritis after the ingestion of *Anabaena, Microcystis* and/or *Aphanizomenon* spp. were documented by Dillenby and Dehnel in 1960. The illnesses were reported in Saskatchewan during the summer of 1959, by people who had been swimming or had fallen into Gull Lake, Echo Lake or Long Lake. Researchers in England recorded cases of pneumonia which occurred after swallowing water which contained toxic *Microcystis aeruginosa* (Turner et al. 1990). There have also been cases of contact irritations or dermatitis reported after swimming in waters containing toxic blue-green algal cells or released toxins (Billings 1981). Other cases of human illness after exposure to or ingestion of blue-green algal toxins have been reported but few details were provided (Bourke and Hawkes 1983).

2.1.3.1 Hepatotoxins

In documented animal poisoning cases, injection or ingestion of algal cells which have produced the polypeptide hepatotoxins, results in hemorrhagic necrosis of the liver. At lethal doses, death occurs within 1 to 2 hours. The actual cause of death is haemorrhagic shock, caused by a pooling of blood in the liver, resulting from destruction of the endothelial lining of the liver sinusoids and disintegration of hepatocyte membranes (Falconer et al. 1981). At sublethal doses, dermal irritation and gastrointestinal, neuromuscular, respiratory or cardiovascular problems may occur.

Falconer et al. (1983a) reported evidence of chronic liver damage in residents of an Australian community whose drinking water supply was severely contaminated with toxic cyanobacteria. They concluded that the hepatotoxins produced by the algae cause elevated amounts of the hepatic enzyme gamma-glutamyltranspeptidase in the plasma of the residents. An outbreak of severe hepatoenteritis, attributed to cyanobacterial toxin, was reported for another Australian community (Hawkins et al. 1985).

2.1.3.2 Neurotoxins

Anatoxin-a is a potent neuromuscular blocking agent similar to acetylcholine. The neurotoxin possesses both muscarinic and nicotinic activity. Muscarinic action can result in the paralysis of the peripheral skeletal muscles, while nicotinic action leads to paralysis of the respiratory muscles. Injection or ingestion of algal cells containing lethal doses of the alkaloid neurotoxin results in death due to respiratory arrest within 15 minutes to 2 hours (Carmichael et al. 1975). Anatoxin-a(s) is a more potent toxin and has different pharmacological properties than those of anatoxin-a. Anatoxin-a(s) is an anticholinesterase which causes symptoms of intoxication that are due to reduced cholinesterase activity and an increase in free acetylcholine in the brain (Mahmood and Carmichael 1986b). The overall effect produces symptoms similar to those caused by anatoxin-a and death is again due to respiratory arrest.

Acute toxicity data for hepatotoxins and neurotoxins which could be found in prairie lakes are summarized in Table 2.2.

2.1.4 Environmental Properties of Toxins

Knowledge of the environmental properties of the cyanobacterial toxins is extremely limited. The polypeptide toxins produced by *Microcystis aeruginosa* and *Anabaena flos-aquae* have been reported to have limited persistence in the aquatic environment (Carmichael and Gorham 1981).

Table 2.2 Documented Toxicity Data

Hepatotoxins

In vivo microcystin LR: LD50 (i.p.* mouse) 50 μg/kg[1]

In vivo microcystin RR: LD50 (i.p.* mouse) 600 μg/kg[1]

In vitro Ames mutagenicity test: negative[2]

In vitro multigene sporulation assay: negative[2]

In vitro chromatid breakage in human lymphocytes: positive[2]

Neurotoxins

In vivo anatoxin-a: LD50 (i.p.* mouse) 200 μg/kg[1]

In vivo anatoxin-a(s): LD50 (i.p.* mouse) 50 μg/kg[1]

In vivo aphantoxin I: LD50 (i.p.* mouse) 10 μg/kg[1]

In vivo aphantoxin II: LD50 (i.p.* mouse) 10 μg/kg[1]

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* intraperitoneally
(the above information was adapted from the following sources:)
1. Carmichael 1988.
2. Repavich et al. 1990.

Degradation of the polypeptide compounds may occur through hydrolysis, photolysis or biodegradation, but these patterns are not well documented.

Berg et al. (1987) studied the degradation of the microcystin toxins in a batch study using 40 g of cells collected from a lake in Wisconsin. Toxicity of the water phase after release of toxin from the algal cells was monitored over a period of 40 days. Work into the stability of the purified microcystin LR has been reported by the United States Army (Wannemacher 1989). In these studies, microcystin LR was resistant to acidic conditions, alkaline conditions and heat up to 300 °C.

2.2 Relevance of Toxins to Drinking Water Sources

Many communities in western Canada depend on freshwater lakes for their raw water supply. During the summer months many of these lakes develop algal blooms. The three study lakes: Coal Lake which serves the City of Wetaskiwin, Driedmeat Lake which serves the City of Camrose and Little Beaver Lake which serves the Village of Ferintosh, all had dense algal blooms during the summer of 1990. Other communities on the prairies often have to deal with similar problems: Moose Lake is the raw water source for the town of Bonnyville, Skeleton Lake for Boyle, Bonnie Lake for Vilna, Buffalo Pound for both Regina and Moose Jaw, Killarny Lake for Killarny, and Lake Minnewasta for Morden.

There are also a large number of prairie farms which depend on dugouts for all or a portion of their domestic water supplies. In 1988 alone, 11,000 farm dugouts were constructed with the assistance of Prairie Farm Rehabilitation Administration and 24% of these were used for domestic water supplies (Lien, PFRA 1990). Many of these dugouts have periodic water quality problems because of poor binding of the essential nutrient phosphorus in the bottom sediments (Riley and Prepas 1984) and nutrient loading from runoff which may be contaminated with fertilizers and animal wastes (Codd et al. 1989).

Blue-green algal blooms are a naturally occurring phenomenon in lakes on the Canadian prairies. These blooms may be accelerated due to land clearing, application of fertilizers, poor waste disposal procedures and urbanization (Skulberg et al. 1984; Codd et al. 1989; Carmichael 1988). As a consequence, toxic algal blooms may become more frequent and many more drinking water supplies may be affected.

2.2.1 Occurrence

The precise conditions which will support the rapid growth of a toxic bloom are not completely understood, but certain conditions favour bloom formation. These include: moderate to high levels of nitrogen and phosphorus; water temperatures between 15° and 30°C; calm or stagnant water; and a pH between 6 and 10 (Skulberg et al. 1984).

The incidence of toxic populations of cyanobacteria in Alberta are widespread, but most cases have not been well documented. The unpredictable occurrence and the variable persistence of the toxic blooms has been a significant handicap for researchers. The greatest contribution to the study of such events on the Canadian prairies was by Paul Gorham during the sixties and seventies. He studied toxic algal blooms in Alberta which include those found at Hastings Lake (Gorham 1964), Disney Lake, Beaverhill Lake (Carmichael and Gorham 1978), Upper Mann Lake, Floating Stone Lake, Smoky Lake, Matchayaw Lake and Lac La Nonne (Gorham et al. 1982).

2.2.2 Detection

Early studies into the cyanobacterial toxins relied on the mouse bioassay as an indicator of the relative toxin levels in samples collected or cultured (Gorham 1960; Gorham et al. 1964). The mouse bioassays were found to be so time consuming and expensive that researchers attempted to develop biochemical assays to monitor the presence of the toxins. Eventually, chemical assay methods were developed for use after initial mouse bioassay screening. Current methods of detection rely on advanced analytical techniques such as high performance liquid chromatography and gas chromatography/mass spectrometry.

2.2.2.1 Instrumental Analysis

Several HPLC methods for the separation of microcystins have been developed and each was designed for use after an initial extraction and clean-up procedure. Harada et al. (1988) outline a procedure which begins with the extraction of the lyophilized cells with 5% acetic acid for 30 min, repeated three times. After centrifugation at 9300g the supernatant is passed through a disposable C18 solid phase extraction cartridge and washed with water and water : methanol (2:8, v:v). The toxins are eluted with methanol and the mixture obtained is evaporated to dryness. HPLC separations of the toxins can be accomplished under reverse-phase isocratic conditions with a C18 column and a mobile phase of methanol : 0.05% trifluoroacetic acid (6:4). The flow rate should be 1 mL/min and UV absorbance (at 238 nm) is used as the detector. An eluent of acetonitrile : 0.01 M ammonium acetate (26:74) is also commonly used (Gathercole and Thiel 1987). Another HPLC method utilizes acetonitrile : 0.10 M potassium dihydrogenphosphate (12:88) with a recently developed internal surface reversed phase column (ISRP) manufactured by Regis Pinkerton (Meriluto and Eriksson 1988). Pure samples of each toxin (generally isolated and purified from well characterized strains of cyanobacteria) are used as standards for the retention time of each toxin.

Gas chromatography/mass spectrometry (GC/MS) methods have recently been developed for the rapid analysis of anatoxin-a (Smith and Lewis 1987). The sensitivity of the GC/MS method is greatly improved when the anatoxin-a is derivatized prior to analysis (Ross et al. 1989). The acetyl derivative of the neurotoxin is formed more easily and reliably than other common derivatives such as the t-butyldimethylsilyl form (Himberg 1989). A number of other analytical methods for the determination of anatoxin-a levels have also been reported. Stevens and Krieger (1988) developed a gas chromatography procedure which utilized the trichloroacetyl derivative of anatoxin-a and electron capture detection. HPLC methods have also been developed to detect anatoxin-a with both normal phase (Wong and Hindin 1982) and reverse phase separations (Astrachan and Archer 1981).

2.2.2.2 Bioassay Methods

The most widely used bioassay method utilizes rats or mice injected intravenously or intraperitoneally with acutely toxic doses of cyanobacteria cells extracts (Krishnamurthy et al. 1986). The animals die within 1 to 3 hours when hepatotoxins have been injected. Death can occur within minutes if the animals have been injected with neurotoxins (Carmichael and Gorham 1978).

Theiss et al. (1988) reported that the immediate cause of death of mice injected with microcystin LR was a loss of hepatic architecture and necrosis. This damage results in a pooling of blood in the liver and a corresponding increase in liver mass. Falconer et al. (1981) also reported an increase in liver mass as well as an increase in serum enzymes after treatment with microcystin toxin. Hermansky et al. (1990) demonstrated that there was a 45% increase in liver weight 60 min after treatment with a suspension having a concentration of 100 μg/L of microcystin LR. They also studied the serum enzyme activities of lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotranferase (ALT). In these assays they found that increases in all three enzymes were statistically significant 45 minutes after treatment with a 100 μg/L suspension of microcystin LR.

2.2.2.3 Biochemical Methods

In a study by Carmichael and Bent (1981) the toxic extracts from algal blooms of *Microcystis aeruginosa* and *Anabaena flos-aquae* agglutinated red blood cells. Agglutination determination consists of: (i) washing lyophilized cyanobacteria cells with physiological saline (0.9% NaCl), (ii) centrifuging the suspension and then mixing portions of the cell-free supernatant with equal volumes of saline-washed erythrocytes in V-shaped microtiter plates, (iii) allowing the mixture to stand for 3 to 4 h and (iv) scoring the presence of toxins as indicated by blood cell agglutination. However, Runnegar and Falconer (1982) reported no red blood cell agglutination with extracts of a strain of *M. aeruginosa* from Australia. This particular strain was shown to contain only two toxins and both contained the leucine-arginine (LR) combination of L-amino acids. The strains used by Carmichael and Bent were collected in central Alberta and although the toxins present were not identified, they may have differed from those in the Australian strain.

2.2.2.4 Odour Correlation

There are a number of genera of cyanobacteria which are known to produce extremely odorous compounds. These include *Microcystis, Anabaena* and *Aphanizomenon* (Palmer 1980). Compounds identified as being produced by these cyanobacteria include geosmin, 2-methylisoborneol (2-MIB), sesquiterpenes, β-cyclocitral, isoamyl alcohol and 6-methyl-5-hepten-2-one (Hayes and Burch 1989).

Waters containing blue-green algal blooms seldom contain only one species of the algae and therefore the odorous compounds with the lowest odour threshold will often mask the odour of other volatile compounds which are present. The most potent, commonly found odorous compounds are geosmin with an odour threshold concentration of 4-20 ng/L and 2-MIB with an odour threshold concentration of 7-20 ng/L (Mallevialle and Suffet 1987; Persson 1980). The odour of compounds such as geosmin and 2-MIB have been associated with the presence of cyanobacteria (Izaguirre et al. 1982; Tabachek and Yurkowski 1976) and correlations between the presence of these compounds and populations of cyanobacteria have been developed (Cees et al. 1974; Rizet and Mouchet 1982). Cyanobacteria reported to produce geosmin and 2-MIB are listed in Tables 2.3 and 2.4.

Microcystis spp. are reported to produce odour causing agents such as β-cyclocitral, isoamyl alcohol, hexanol, 6-methyl-5-hepten-2-one and phenyl ethanol (Hayes and Burch, 1989). Jüttner (1988) reported that *Microcystis* is characterized by an ability to produce large quantities of β–cyclocitral, which has a threshold odour concentration of 19 μg/L (Persson and Jüttner 1983). Herrmann and Jüttner (1977) also reported the isolation of 2-aminopropane from waters containing *Microcystis* spp. Jenkins et al. (1967) found that *Microcystis flos-aquae* also produced isopropyl mercaptan (sulfur odour).

Table 2.3 Reported Geosmin Producing Cyanobacteria

Genus	Species	Reference (adapted from)
Anabaena	*macrospora*	Ashitani et al. 1988
	flos-aquae	Hayes and Burch 1989
	scheremetievi	Izaguirre et al. 1982
	circinalis	Persson 1983
	species	Slater and Blok 1983
Aphanizonmenon	*flos-aquae*	Matsumoto and Tsuchiya 1988
	gracile	Jüttner et al. 1986
	species	Slater and Blok 1983
Lyngbya	*aestuarii*	Tabachek and Yurkowski 1976
Oscillatoria	*brevis*	Berglind et al. 1983
	agardhii	Berglind et al. 1983
	bornetii	Berglind et al. 1983
	tenuis	Medsker et al. 1968
	simplicissima	Izaguirre et al. 1982
	amoena	Matsumoto and Tsuchiya 1988
	splendida	Matsumoto and Tsuchiya 1988
	cortiana	Persson 1983
	prolifica	Persson 1983
	variablilis	Persson 1983
	species	Matsumoto and Tsuchiya 1988
Phormidium	*tenue*	Ashitani et al. 1988
	species	Berglind et al. 1983
Symploca	*muscorum*	Medsker et al. 1968
Schizothrix	*muelleri*	Persson 1983

Table 2.4 Reported MIB Producing Cyanobacteria

Genus	Species	Reference (adapted from)
Lyngbya	*cryptovaginata*	Persson 1983
Oscillatoria	*curviceps*	Izaguirre et al. 1983
	tenuis	Izaguirre et al. 1982
	brevis	Berglind et al. 1983
	geminata	Matsumoto and Tsuchiya 1988
	limntica	Matsumoto and Tsuchiya 1988
	species	Matsumoto and Tsuchiya 1988
Phormidium	species	Berglind et al. 1983

2.2.3 Control and Treatment Methods

Summer months on the prairies are ideal for the growth of cyanobacteria and often they outcompete all other species of algae. Prevention of algal blooms may be possible with nutrient control and proper reservoir management. When prevention has not been possible, an algal bloom results in water quality deterioration and treatment for removal of the algae becomes critical.

2.2.3.1 Bloom Control

Copper sulfate has been used extensively for the control of algal blooms since 1905 (McKnight et al. 1983). In Alberta, the recommended copper sulfate treatment concentration for farm dugouts which have algal blooms, is 50-100 µg/L Cu (Alberta Agriculture 1989). Much higher concentrations were previously used in many areas, although the lower concentrations are now known to be effective (Whitaker et al. 1978). Copper sulfate can be an effective agent for short-term control of blue-green algal species, but there are a number of problems related to its use in the treatment of algal blooms (Hanson and Stefan 1984). Blue-green algae often develop into more resistant strains with long-term copper sulfate treatment. In addition to this the short-term usage is not always reliable for all algal species and the treatment does not attack the cause of the problem (nutrients), but instead treats a symptom. It has also been reported that the use of higher than recommended concentrations of copper sulfate can kill the zooplankton which graze on some species of algae and result in increased algal levels (Peterson and Swanson 1988). Another problem associated with the copper sulfate treatment, is the possible release of cyanobacterial toxins to the water phase as the cells die (Berg et al. 1987). The copper sulfate does not reduce the toxicity of these compounds and natural detoxification through biodegradation may take up to 40 days.

An alternate treatment method currently being studied involves the application of calcium carbonate or calcium hydroxide (Prepas et al. 1990). Calcium hydroxide treatment has been shown to slightly reduce the levels of chlorophyll α (which is an indicator of the algal biomass) during the summer of treatment, and can result in substantial decreases in chlorophyll α levels in subsequent years. This is thought to be due to a long term decrease in the total phosphorus (Murphy et al. 1990). This method of treatment controls the nutrient supply instead of killing the algal cells. Consequently, subsequent algal blooms should not occur, and a sudden, uncontrolled release of toxic compounds to the water would not be expected.

2.2.3.2 Removal or Destruction by Water Treatment

Prolific algal growth in fresh waters causes further difficulties for drinking water treatment facilities. Rapid algal growth can result in aesthetically unacceptable water due to colour, turbidity, or odour problems. Algal cells can also lead to filter clogging problems, increased disinfectant demands and the presence of cyanobacterial toxins.

The basic coagulation-filtration-chlorination treatment process was shown to be ineffective in the removal of the toxins (Hoffman 1976). In this study, toxin concentrations of 10 mg/L were used to determine the removal efficiencies of a series of water treatment processes. Mouse bioassays were used to determine the amount of toxin remaining in the water after each stage of treatment. Tests after prechlorination, lime addition, flocculation and sedimentation, sand filtration and postchlorination indicated that toxins were not removed by any of these processes. But, adsorption with granular activated carbon (GAC) or a 100 mg/L dose of powdered activated carbon (PAC) was effective in removing the toxin.

Falconer et al. (1983b and 1989) also found that activated carbon adsorption was effective in toxin removal, using either PAC addition or GAC filtration. A number of brands of PAC and GAC were tested for the removal of hepatotoxins and neurotoxins in both laboratory and pilot plant studies. They also reported that desorption of the toxins was not detected under laboratory conditions designed to promote toxin release from the GAC filters. More detailed study into the various treatment alternatives was performed in Finland (Keijola et al. 1988; Himberg et al. 1989). In these studies, both laboratory and pilot scale experiments were carried out to study the effect of flocculation, coagulation and slow sand filtration, as well as combined processes such powdered activated carbon addition before flocculation, activated carbon filtration and flocculation, chlorination after flocculation, and ozonation before flocculation. HPLC and GC/MS methods were used to monitor the toxin removal efficiencies.

In the laboratory scale experiments (Himberg et al. 1989), GAC after flocculation completely removed the hepatotoxins being monitored and removed 94% of anatoxin-a. Ozonation (1 mg/L) prior to flocculation also completely removed the hepatotoxins and removed 96% of the anatoxin-a. In pilot plant investigations using fresh *Microcystis aeruginosa* bloom material (Keijola et al. 1988), PAC (20 mg/L) proved to be effective in removing 99% of the hepatotoxin being monitored. An ozone dose of 1 mg/L was sufficient to remove 90% of the hepatotoxin. A second pilot plant experiment using freeze dried material corresponded to a situation where a bloom had been treated with $CuSO_4$ or had collapsed due to old age, whereupon the toxin was readily released to the water phase. Preozonation removed only 50% of the hepatotoxin, while 20 mg/L of PAC removed 90% of the toxin. PAC doses of 100 mg/L and 200 mg/L completely removed the toxin when fresh or freeze-dried bloom material was used. Such PAC dosages would be impractical at most water treatment plants.

The reported toxin removal efficiencies of the various water treatment processes are summarized in Table 2.5.

Table 2.5 Toxin Removal Efficiencies of Various Water Treatment Processes.

Study Type	Process Sequence	Toxin Removal Efficiency	Comments	Reference (adapted from)
Laboratory			Pure (unspecified) toxin added to water	Hoffman 1976
		qualitative only	toxins monitored by mouse bioassay	
	A 1) GAC filtration	removed (no death)	GAC type:	
			Pittsburgh Filtrasorb 3000	
	B 1) chlorination	not removed (death)	CaOCl dose 5 mg/L	
	2) FeCl3 flocculation		FeCl3 dose 20 mg/L	
	3) sedimentation	not removed (death)		
	4) sand filtration	not removed (death)		
	5) GAC filtration	removed (no death)		
	C 1) lime addition	not removed (death)	lime dose 50 mg/L	
	2) FeCl3 flocculation		FeCl3 dose 20 mg/L	
	3) sedimentation	not removed (death)		
	4) sand filtration	not removed (death)		
	5) chlorination	not removed (death)	CaOCl dose 5 mg/L	
	6) GAC filtration	removed (no death)		
	D 1) PAC addition		PAC type:	
	(10mg/mg toxin)	not removed (death)	Darco Grad KB	
	2) PAC addition			
	(100 mg/mg toxin)	removed (no death)		
Laboratory			freeze-dried cyanobacterial cells	Falconer et al. 1989
			toxins monitored by mouse bioassay	
	A 1) GAC filtration	100% neurotoxin	AC types listed in order of decreasing	
	neurotoxin trials		removal efficiency	
			1. Norit ROW 0.8 Supra	
			2. Calgon Filtrasorb 200	
			3. Calgon Filtrasorb 100	
			4. Taiko CW 820A	
			5. Kintal 6, 16J	

....Continued

Table 2.5 Toxin Removal Efficiencies of Various Water Treatment Processes (continued).

Study Type	Process Sequence	Toxin Removal Efficiency	Comments	Reference (adapted from)
Laboratory	B 1) GAC filtration (hepatotoxin trials)	100% hepatotoxin	1. Calgon Filtrasorb 100 2. Darco granular 3. Calgon Filtrasorb 200 4. Norit ROW 0.8 Supra 5. Norit PK1-3 6. Taiko CW 120 7. Taiko CW 120 (used) 8. Taiko Cw 820B 9. Kintal 6, 16J 10. Taiko CW 820A	Falconer et al. 1989
Pilot Plant			freeze-dried cyanobacterial cells toxins monitored by mouse bioassay	Falconer et al. 1989
	A 1) PAC addition (neurotoxin trials) 2) alum flocculation 3) polyelectrolyte	100% neurotoxin	PAC 80 mg/L alum dose 30 mg/L polyelectrolyte (LT25) 0.05 mg/L 1. Darco HDB 2. Norit SA5 3. Calgon GW	
	B 1) PAC addition (neurotoxin trials) 2) alum flocculation	100% neurotoxin	PAC 80 mg/L alum dose 30 mg/L 1. Norit SA5 2. Calgon GW 3. Darco HDB	
	C 1) GAC filtration (neurotoxin trials) 2) rapid sand filtration	100% neurotoxin	1. Norit ROW 0.8 Supra 2. Calgon Filtrasorb 100 3. sand only	

....Continued

Table 2.5 Toxin Removal Efficiencies of Various Water Treatment Processes (continued).

Study Type	Process Sequence	Toxin Removal Efficiency	Comments	Reference (adapted from)
Pilot Plant	D 1) GAC filtration (hepatotoxin trials)			Falconer et al. 1989
	2) rapid sand filtration	100% hepatotoxin	1. Norit ROW 0.8 Supra 2. Calgon Filtrasorb 100 3. Darco granular 4. Taiko CW120 (#1) 5. Norit PK1-3 6. Taiko CW120 (#2 used) 7. Taiko CW120 (#2) 8. Kintal 6, 16J 9. sand only	
Pilot Plant			fresh cyanobacterial bloom material and freeze-dried cyanbacterial cells toxins monitored by HPLC, GC/MS results not well detailed	Keijola et al. 1988
	A 1) ozonation	90% (fresh bloom) 50% (freeze-dried)	ozone dose 1-1.5 mg/L	
	2) alum flocculation		alum dose 62-75 mg/L	
	3) sand filtration	90% (freeze-dried)		
	B 1) PAC addition	99% (fresh bloom) 90% (freeze-dried) 100% (freeze-dried)	PAC 20 mg/L, type: Merck analytical PAC 100 mg/L	
	2) alum flocculation		alum dose62-75 mg/L	
	3) sedimentation			
	4) sand filtration			
	C 1) slow sand filtration (biologically active)	68-74% neurotoxin 82-86% hepatotoxin	filtration rate 0.085 m/h	

....Continued

Table 2.5 Toxin Removal Efficiencies of Various Water Treatment Processes (concluded).

Study Type	Process Sequence	Toxin Removal Efficiency	Comments	Reference (adapted from)
Laboratory			lyophilized cyanobacteria cells	Himberg et al. 1989
			toxins monitored by HPLC	
	A 1) alum flocculation		alum dose 36-41 mg/L	
	2) sand filtration			
	3) chlorination	11-32% hepatotoxin		
		0-14% neurotoxin		
	B 1) FeCl3 flocculation		FeCl3 dose 55 mg/L	
	2) sand filtration			
	3) chlorination	<0-16% hepatotoxin		
		0-49% neurotoxin		
	C 1) PAC addition		PAC dose 5 mg/L	
	2) alum flocculation		alum dose 71-79 mg/L	
	3) sand filtration			
	4) chlorination	13-34% hepatotoxin		
		58-82% neurotoxin		Keijola et al. 1988
	D 1) alum flocculation		alum dose 66-67 mg/L	Himberg et al. 1989
	2) sand filtration			
	3) GAC filtration		GAC type:	
	4) chlorination	100% hepatotoxin	B. Jakobssons Emb & Kemi AB	
		94-97% neurotoxin		
	E 1) ozonation		ozone dose 1 mg/L	
	2) alum flocculation		alum 74-90 mg/L	
	3) sand filtration			
	4) chlorination	100% hepatotoxin		
		96-100% neurotoxin		

3. RESEARCH OBJECTIVES

The three main objectives of this research were established to obtain basic information on the biogenic compounds produced by cyanobacteria in some prairie lakes and reservoirs during the summer of 1990. This work covers the preliminary research for a longer term project.

3.1 Survey of Toxin Occurrence

The primary objective of this research was to survey a number of raw water supply lakes and agricultural dugouts in Alberta in order to establish the prevalence of toxic blue-green algal blooms. Water bodies which are used as raw water supplies were selected for study because of their immediate relevance to human health. Recent advances in analytical techniques made it possible to provide accurate and sensitive measurements of the algal toxins. Earlier work in Alberta had to rely upon mouse bioassays for detecting the presence of toxins.

3.2 Evaluation of Odour for Detection of Toxin

The second objective of this study was to evaluate the existence of any basic correlation between odour levels and toxin production and the reliability of any correlation as a general method of toxin avoidance.

3.3 Evaluation of Toxin Release by Bloom Control Measures

The final objective of this study was to monitor the amounts of toxin released from algal cell biomass to the water phase following treatment with copper sulfate or calcium hydroxide (lime). Copper sulfate is a chemical commonly used to control algal blooms and lime has been proposed for an alternate treatment.

4. METHODS AND MATERIALS

4.1 Field Procedures

During each sampling visit the water temperature, the water depth, and the Secchi depth were measured at the chosen sampling point. The Secchi depths were measured with a 20-cm Secchi disk.

4.1.1 Sampling

All samples were collected from the approximate centre of the water body. Water samples were collected at a measured depth of 1-3 meters with weighted Tygon® tubing and were stored at 4 °C for a maximum of 24 h before analysis. Samples for phosphorus and chlorophyll α were stored in opaque Nalgene bottles, and samples for odour analysis were stored in acid-washed (H_3PO_4) borosilicate glass bottles.

All algal samples were collected and concentrated with a 80-µm Nitex® mesh conical net which was 90-cm long with a 29-cm diameter aperture, and fitted with a bucket made with 64-µm mesh (Figure 4.1). Algal samples were stored at 4 °C for up to 48 h in borosilicate glass. Samples for algal typing were stored in clear acid-washed (3% HCl) borosilicate glass bottles and the concentrated algal samples for toxin analysis were stored in borosilicate glass bottles which had been soaked in 3% NaOCl and then rinsed 7 times with distilled water. The lids for all glass storage bottles were lined with Teflon®.

4.1.2 Odour Evaluation

Each water sample was also checked for the odour of three target odorous compounds; geosmin, 2-methylisoborneol and β-cyclocitral (Figure 4.2). For the dugout program, the odour of the water was checked when it

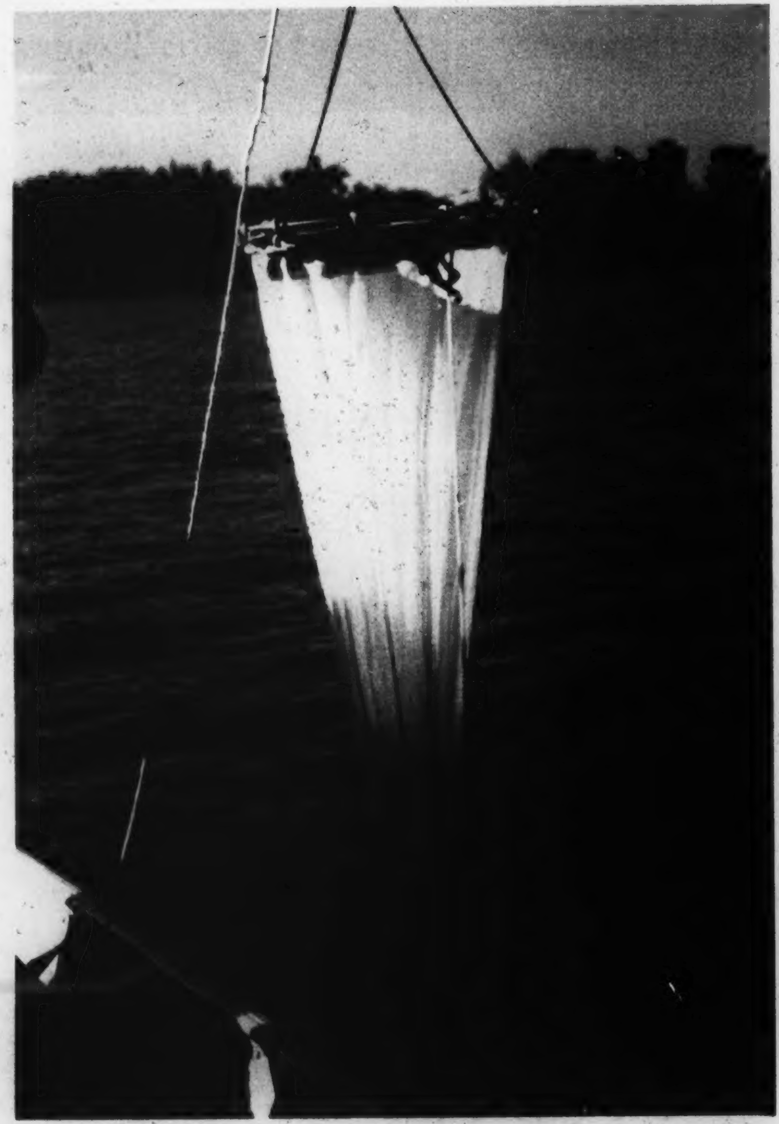
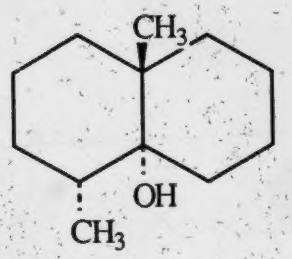
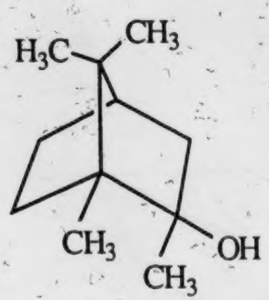


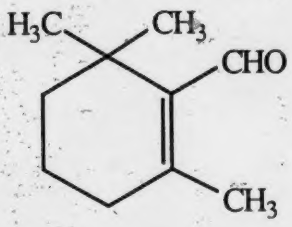
Figure 4.1 Conical Net Used in Algal Sampling



Structure of geosmin


H3C
CH3
CH3
OH
CH3

Structure of 2-methylisoborneol


H3C
CH3
CHO
CH3

Structure of β-cyclocitral

Figure 4.2 Chemical Structures of Target Odour Compounds

was sampled and if a noticeable odour was detectable, a 2-L sample was collected. When sampling at very distant locations, a hexane extraction was carried out on the 2-L sample in the field according to the method developed by Brownlee et al. (1984). 10-mL of double distilled hexane was added to the 2-L water sample and the mixture was vigorously shaken for 5-min. The upper hexane layer was then removed and $NaSO_4$ was added to remove any water present in the hexane. The odour of the extract was then compared to standards of the odorous geosmin and 2-methylisoborneol.

For the lake water program, samples were collected regardless of whether an odour was perceptible. These samples were collected in 4-L borosilicate glass bottles and were stored at 4 °C during transport to the laboratory. The samples were analysed as described in section 4.3.3.

4.2 Sample Preparation

The concentrated algal samples were decanted and filtered through a coarse filter to remove debris within 48 h. The samples were then transferred to detoxified (3% NaOCl) round bottom flasks. The algal cell suspensions were frozen in an acetone-dry ice bath and then were freeze-dried with a Labconco 4.5-L benchtop freeze-drier.

4.3 Analysis

4.3.1 Hepatotoxins

The extraction method developed by Harada et al. (1988) was modified slightly for this analysis. Approximately 100 mg dried cells were transferred to a 15-mL centrifuge tube, 5-mL 5% acetic acid was added and the suspension was mixed thoroughly with a vortex. The cell mixture was then left in the dark for at least 1-h before mixing again and sonicating 3 times for 30-s. The mixture was left in the dark for at least 1-h, mixed and centrifuged for 1-h at highest speed. The solution was then transferred to a disposable C18 cartridge (Supelco) which had been prepared by washing with 10-mL methanol and then 10-mL water. After applying the sample the cartridge was washed with 10 mL 5% acetic acid, 10-mL water and 10-mL of 10% methanol in water. The microcystin toxins were then eluted with 5-mL methanol. The methanol was evaporated with a slow nitrogen flow overnight. The residue was then redissolved in 750-µL HPLC solvent (10 mM ammonium acetate : acetonitrile 74 : 26) and transferred to a 1.5-mL Eppendorff tube and centrifuged for 1-h. The supernatant was drawn into a 1-mL Luer lock syringe and passed through a 0.22-µm filter. A 20-µL sample was then injected onto a high performance liquid chromatography system equipped with Waters model 6000A pumps, a Hewlett Packard diode array detector (model 1040A) with U.V. detection at 238 nm and a Hewlett Packard HP85 data processor.

A number of the HPLC hepatotoxin analysis methods outlined in the literature were attempted before an appropriate method was selected. Many HPLC methods are instrument specific and a challenged was faced in making an analysis work with the instrument available. The diode array detector has a small volume flow cell and the phosphate buffer and acetonitrile solvent system used by Meriluto and Eriksson (1988) caused continual pressure increases at the detector which were a result of phosphate salts depositing in the flow cell. Chromatographic separation using the recently developed internal surface reversed phase column, 5 µm GFF ISRP, 15 cm x 4.6 mm ID (Regis Pinkerton) was also attempted. The combined effects of separation through size exclusion and reversed phase chromatography with this column have been shown give adequate resolution of microcystins with a shorter run time than reversed phase alone. The suggested acetonitrile : phosphate buffer solvent system (Meriluto and Eriksson 1988) was once again unsuccessful because of pressure build up. Resolution of standards was achieved using a solvent system of 20 mM ammonium acetate : acetonitrile (74:26), but when field samples were analysed using this method the toxin peaks could not be resolved, because of the numerous additional peaks. Peaks corresponding to toxin standards were resolved in the field samples using a C18 μ Bondapak, 30 cm x 3.9 mm ID (Waters), with 10 mM ammonium acetate : acetonitrile (74 : 26) solvent system at 1 mL/minute. This solvent system could not be used in the analysis of the batch toxin release samples because resolution could not be achieved due to additional large background peaks. A solvent system of 10 mM ammonium acetate : acetonitrile (76:24) at 1.6 mL/min was used to achieve adequate resolution.

Microcystin LR and RR standards were obtained from Dr. W.W. Carmichael at Wright State University in Dayton, Ohio. Standards of an equal mixture of the LR and RR were analysed under the HPLC conditions mentioned above. A typical standard chromatogram is shown in Figure 4.3. The areas for the LR and RR peaks were plotted against concentration as shown in Figure 4.4. The standard solution of 25 ppm LR and RR was injected each day before any samples were analysed. A typical injection of an algal cell extract is shown in Figure 4.5.

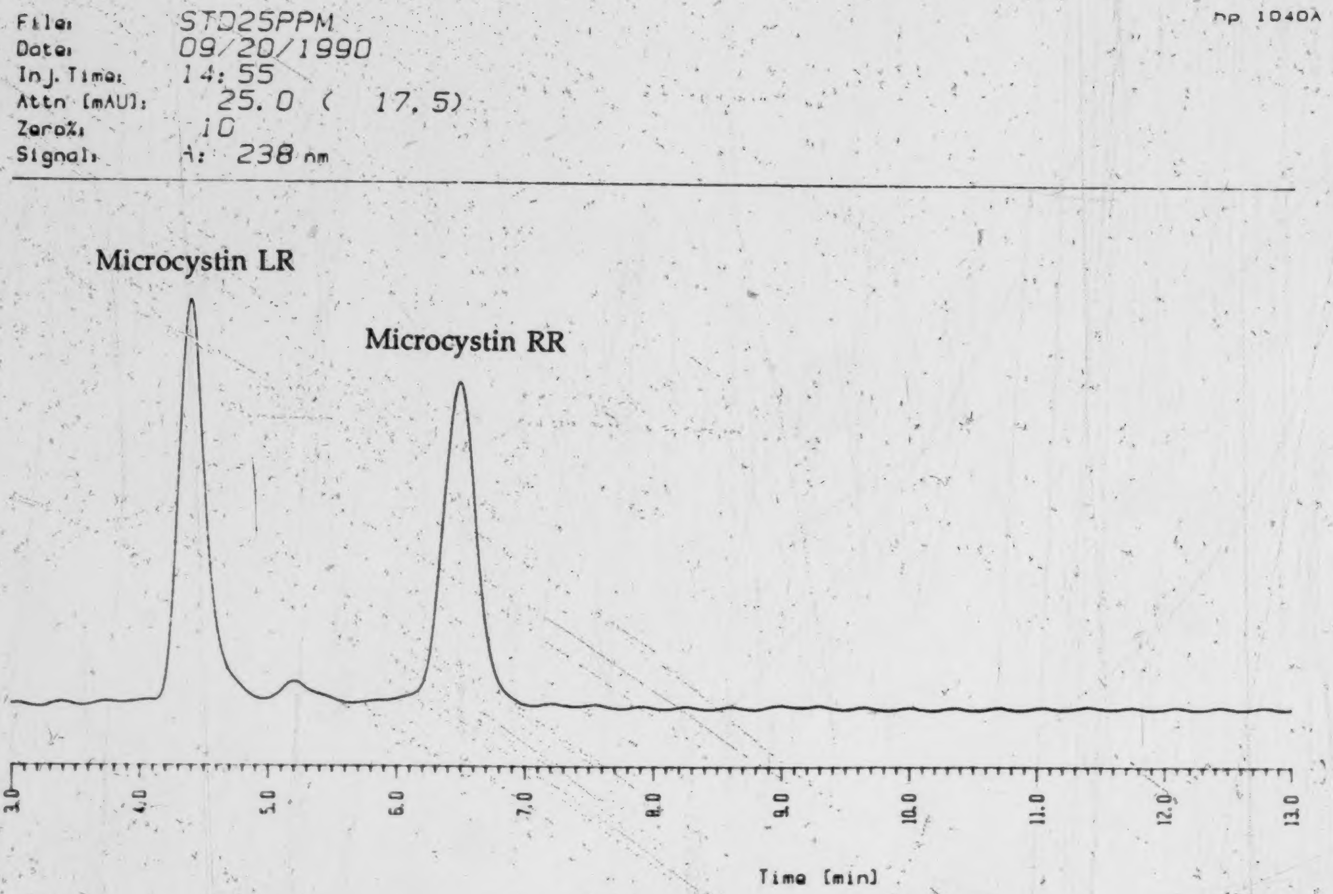


Figure 4.3 Chromatogram of *Microcystis aeruginosa* Toxin Standards Microcystin LR and Microcystin RR

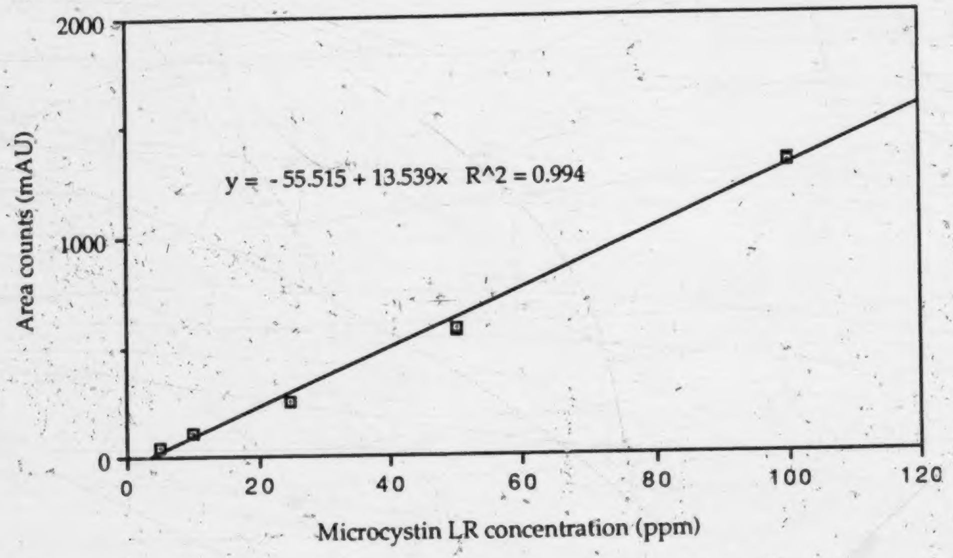


Figure 4.4 Microcystin LR Standard Curve

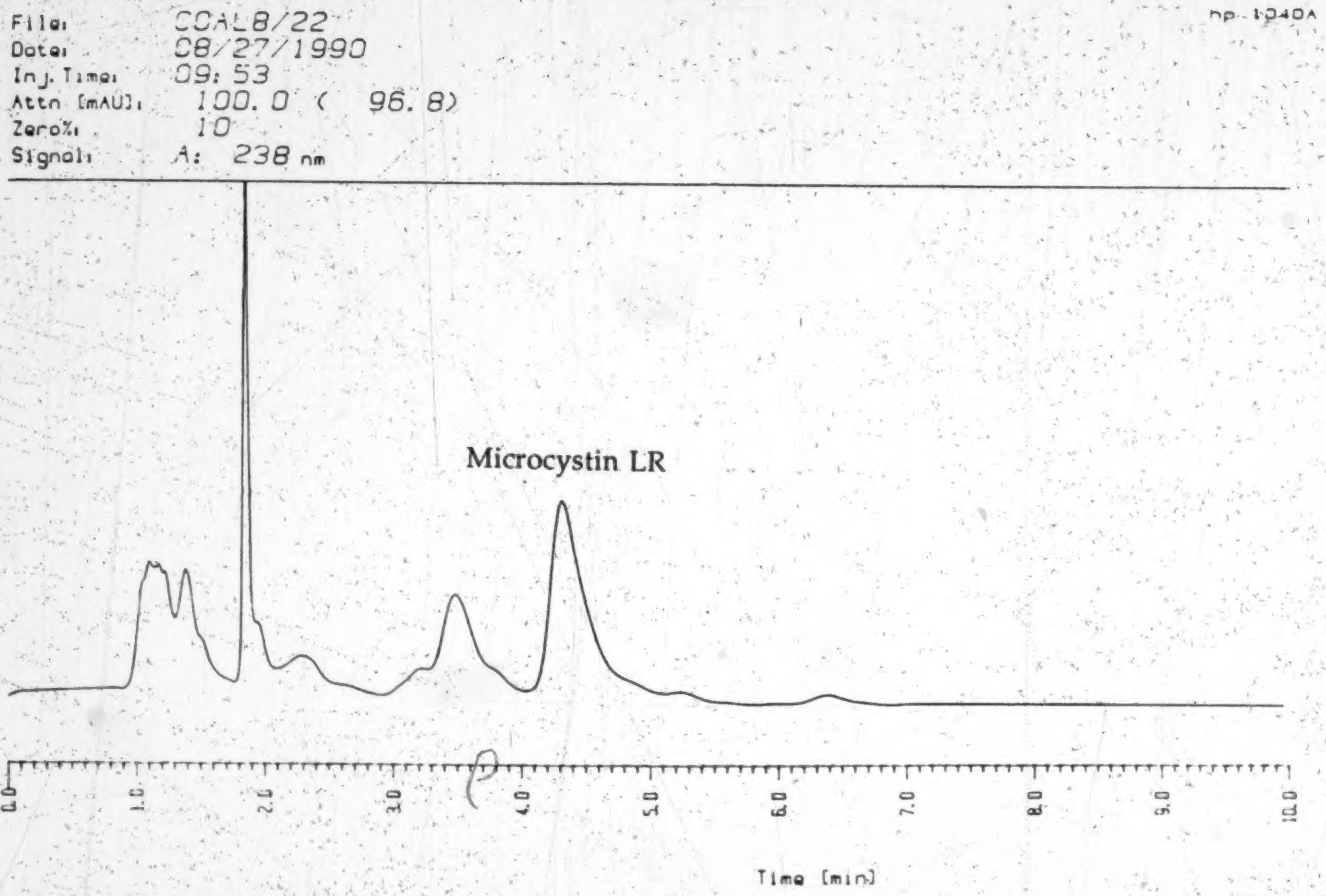


Figure 4.5 Typical Chromatogram of Extract from a Natural Sample
(Coal Lake August 22, 1990)

4.3.2 Anatoxin-a

The extraction method developed by Himberg (1989) was modified slightly for the analysis of the neurotoxin, anatoxin-a. Approximately 50 mg of freeze-dried material was homogenized and placed in a 50-mL centrifuge tube. A 25-mL aliquot of methanol and 0.25-mL of concentrated hydrochloric acid were added, the tubes were capped and the suspension was mixed thoroughly. The algal cells in suspension were lysed in an ultrasonic bath for 15-min and were then centrifuged. The extraction step was then repeated on the centrifuged pellet and the combined supernatants were filtered through a Gelman Acro 50 filter. The solution was then evaporated nearly to dryness under a gentle flow of prepurified nitrogen.

A number of derivatization methods were attempted. The *tert*-butyldimethylsilyl derivatization of anatoxin-a was attempted because this compound has been shown to give good GC separation with unique, abundant high mass fragments (Ross et al. 1989). This derivatization requires a base such as imidazole be present to act both as an acid acceptor and as an intermediary silyl donor as the N-*tert*-butyldimethylsilyl-substituted imidazole (Mawhinney and Madson 1982). Equimolar amounts of anatoxin-a and *tert*-butyldimethylsilyl hydrochloride salt were mixed with twice as many moles of imidazole and heated to 60 °C for 30-min, but high yields of the derivative were not formed. This method is not expected to give high yields when a secondary amine is being derivatized (Mawhinney and Madson 1982) and the preferred derivatizing agent is *tert*-butyldimethylsilyl-N-methyltrifluoro acetamide. However this procedure is too moisture sensitive to be used effectively with field samples. The acetyl derivative was finally chosen, despite poorer GC characteristics and less unique fragment ions, because it was easily formed and was appropriate for field samples. According to the method of Himberg (1989), the extraction residue was dissolved in 1-mL of chloroform and transferred quantitatively to a 10-mL borosilicate culture tube. 1 mL of concentrated acetic acid and 0.5-mL of acetic anhydride were then added, the mixture was mixed gently, and the tube was capped tightly. The mixture was then allowed to react at 50 °C for 16-h. The solution was then evaporated nearly to dryness in a Kuderna-Danish evaporator for nonvolatile solvents. When no smell of acetic acid was detectable the residue was dissolved in 1-mL of chloroform and applied to a Supelclean® silica cartridge. The cartridge was washed with 7.5-mL of acetone-chloroform (5-95) and then the acetylated anatoxin-a was eluted with 2-mL of methanol. The solution was finally concentrated down to 100-µL in a Kuderna-Danish evaporator for nonvolatile solvents and chloroform was added to give a final volume of 1-mL.

This chloroform solution was then used for gas chromatographic-mass spectrometric (GC-MS) analysis for the acetylanatoxin-a. Immediately prior to analysis, 10-µL of 1-acetylpiperidine (50:50 in chloroform) was added as an internal standard. The GC-MS analysis was carried out in the selected ion mode (SIM) on a Hewlett-Packard 5890 gas chromatograph with an HP 5970 mass-selective detector and an HP 59940 Chemstation® data system. The column was 30 m x 0.25 mm I.D. with a DB-1 stationary phase (J & W Scientific) with 0.25-µm film thickness. The detector temperature was 250 °C and the oven was temperature programmed from 80 to 275 °C at 15 °C/min, with a final hold time 4-min to give a total run time of 17-min. A 1-µL injection was made with an HP 7673A auto injector, with an injector pressure of 70 kPa and an injector temperature of 240 °C. Ions of m/z = 207, 165, 164, and 136 were used for monitoring acetylanatoxin-a, and ions of m/z = 84 and 127 were monitored for the internal standard. A typical total ion mass spectra of acetylanatoxin-a is shown in Figure 4.6 and a typical group of SIM traces is shown in Figure 4.7.

Standards of anatoxin-a were obtained from Dr. O.E. Edwards of the Department of Chemistry at Carlton University in Ottawa and a known quantity was derivatized as described above. The internal standard was added and the ratio of the m/z = 165 ion of the acetylanatoxin-a peak area to the m/z = 84 ion of the acetylpiperidine was plotted relative to the mass of anatoxin-a injected (Figure 4.8) Although the m/z = 207 ion was more abundant in the acetylanatoxin-a spectra, it was not selected for the quantitative ratio because it is a common column bleed ion.

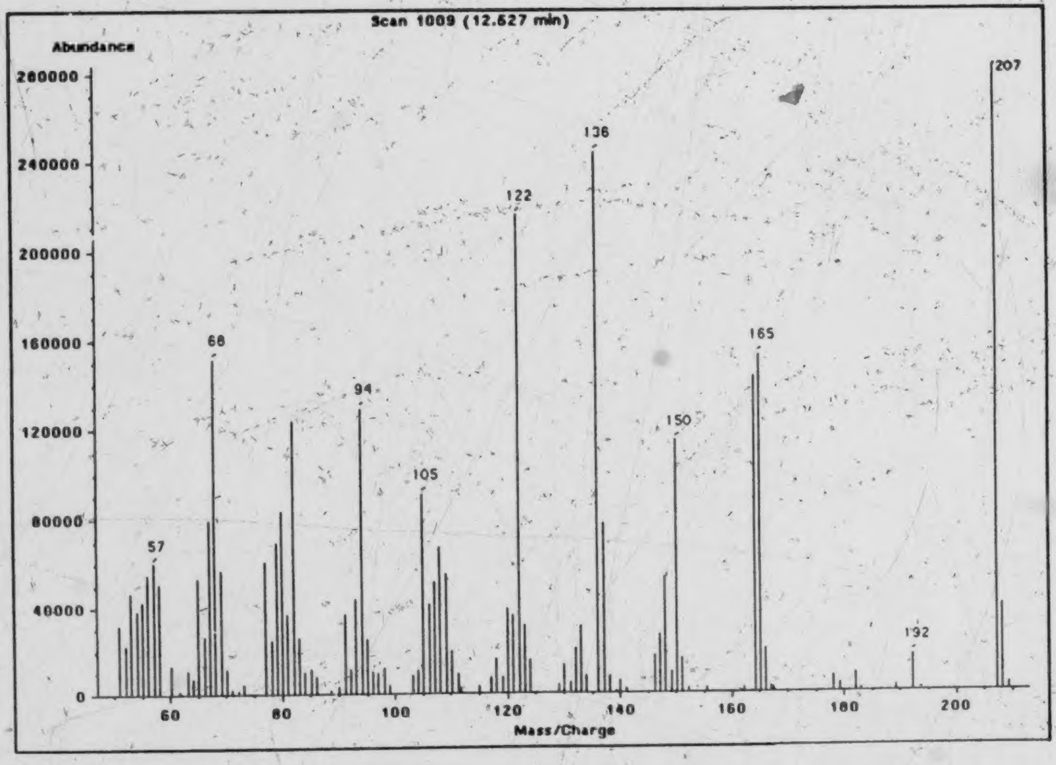


Figure 4.6 Total Ion Mass Spectra of Acetylanatoxin-a Standard

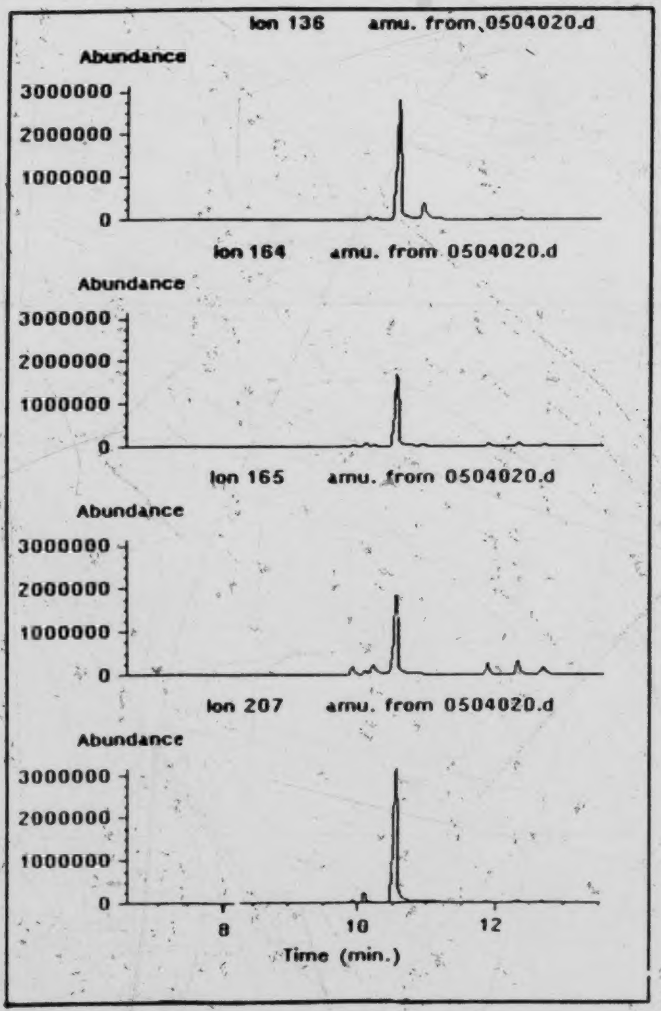


Figure 4.7 Selected Ion Monitored Traces of Acetylanatoxin-a Standard

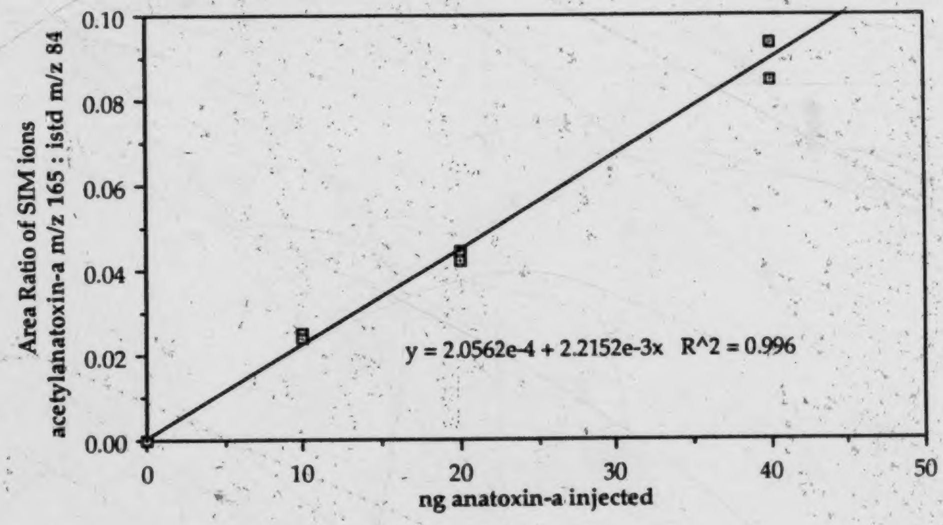


Figure 4.8 Acetylanatoxin-a Standard Curve (acetylpiperidine as internal standard)

4.3.3 Odour Compounds

Analyses were performed by Norine Motkosky according to the base-neutral extraction procedure of the U.S. Environmental Protection Agency analytical method 625 detailed in the Federal Register (1984). Solvent extracts were analyzed by GC and flame ionization detection (FID) and chromatographic sniffing using an HP 5890 GC under the following conditions: injector temperature; 225 °C, detector temperature; 300 °C, column; DB-1 stationary phase (J & W Scientific) with 0.25-µm film thickness, 30 m x 0.25 mm I.D., initial column temperature; 35 °C for 5 min, temperature program rate of 5 °C/minute to 290 °C, final temperature hold; 5 min.

Solvent extracts were subjected to a chromatographic sniffing procedure adapted from the method of Sävenhed et al. (1985). The GC was modified by removing the FID and the flame jet and pushing the column end up 10-cm. A 7-cm length of stainless steel tubing was placed over the column to prevent condensation of components at the end of the column. A glass funnel was placed over this tubing with 0.5-cm of the column protruding. As odorous components of each sample eluted, the time, intensity and first impression descriptor were recorded. Odour reference standards were used to establish retention times and descriptors of target compounds.

4.3.4 Dissolved Organic Carbon

Samples were collected in 250-mL glass bottles, immediately acidified to pH 2 with concentrated sulphuric acid, sealed with Teflon® lined caps and stored at 4°C in the dark until analyzed (APHA, AWWA and WPCF, 1989). Immediately prior to analysis all samples were filtered through 0.45µm cellulose nitrate filters. Analyses were carried out with a Dohrmann DC 80® TOC analyzer which utilizes UV promoted persulfate oxidation followed by

IR detection of resulting carbon dioxide. An autosampler was utilized and all samples were purged for 8-10 min with prepurified nitrogen, to remove most of the volatile inorganic carbon present in each sample. The DC 80 TOC analyzer was calibrated before each set of analysis with 4 injections of a standard potassium hydrogen phthalate solution (prepared fresh weekly) and was checked again during the analysis set with another 4 standard injections. All samples were analyzed in triplicate for total carbon (TC) and inorganic carbon (IC) and the difference in the values was reported as the dissolved organic carbon (DOC). Samples sets demonstrating a relative standard deviation of greater than 2% for DOC were discarded.

4.3.5 Phosphorus

Analyses were performed in the laboratories of Dr. Ellie E. Prepas by Gertie Hutchinson. Samples from the laboratory batch study were collected in 250-mL glass bottles which had been 7 times with double distilled water. The samples were immediately filtered through 0.45-μm HAWP Millipore membrane filters which were pre-rinsed with 500-mL distilled, deionized, phosphorus free water. Filtrates were stored at 4°C before transfer to culture tubes for analysis of total dissolved phosphorus according to the potassium persulfate method (Menzel and Corwin, 1965) within 48-h.

4.3.6 Chlorophyll

Analyses were performed in the laboratories of Dr. Ellie E. Prepas by Mark Serediak and Warren Zyla. Samples were filtered under low pressure (maximum -50 kPa) through Whatman GF/C filters and the filters were placed in petri dishes wrapped in aluminum foil and frozen until analysis. The concentration of chlorophyll α was determined in triplicate with a spectrophotometric technique based on Ostrofsky's ethanol extraction procedure (Bergmann and Peters 1980).

4.3.7 Dissolved Oxygen

Dissolved oxygen measurements were determined with a YSI 54A Oxygen Meter. The meter was initially calibrated with oxygen saturated water using more accurate dissolved oxygen values obtained with the Winkler titration method (APHA, AWWA and WPCF 1989). The meter was calibrated for temperature and barometric pressure daily with an air calibration.

4.3.8 pH

pH values were determined with a Fisher 805 MP digital pH meter. The meter was calibrated through a two point calibration using buffers with pH 4.00 and 10.00 and correcting for temperature.

4.3.9 Species Typing

The various blue-green algal species in each sample collected were determined in the laboratories of Dr. Ellie E. Prepas by Angeline Lam within 24 h of collection using a compound microscope (magnified up to 2000X). Identification of each species was based on descriptions given by Prescott (1978). The relative proportions of predominant species of cyanobacteria in each sample were estimated by cell counts of subsamples under a dissecting microscope. The three predominant blue-green algal species of the lakes studied are pictured in Figures 4.9a, 4.9b and 4.9c

Figure 4.9a Predominant Cyanobacterial Species of Study Lakes *Microcystis aeruginosa* (Magnification 1000x)

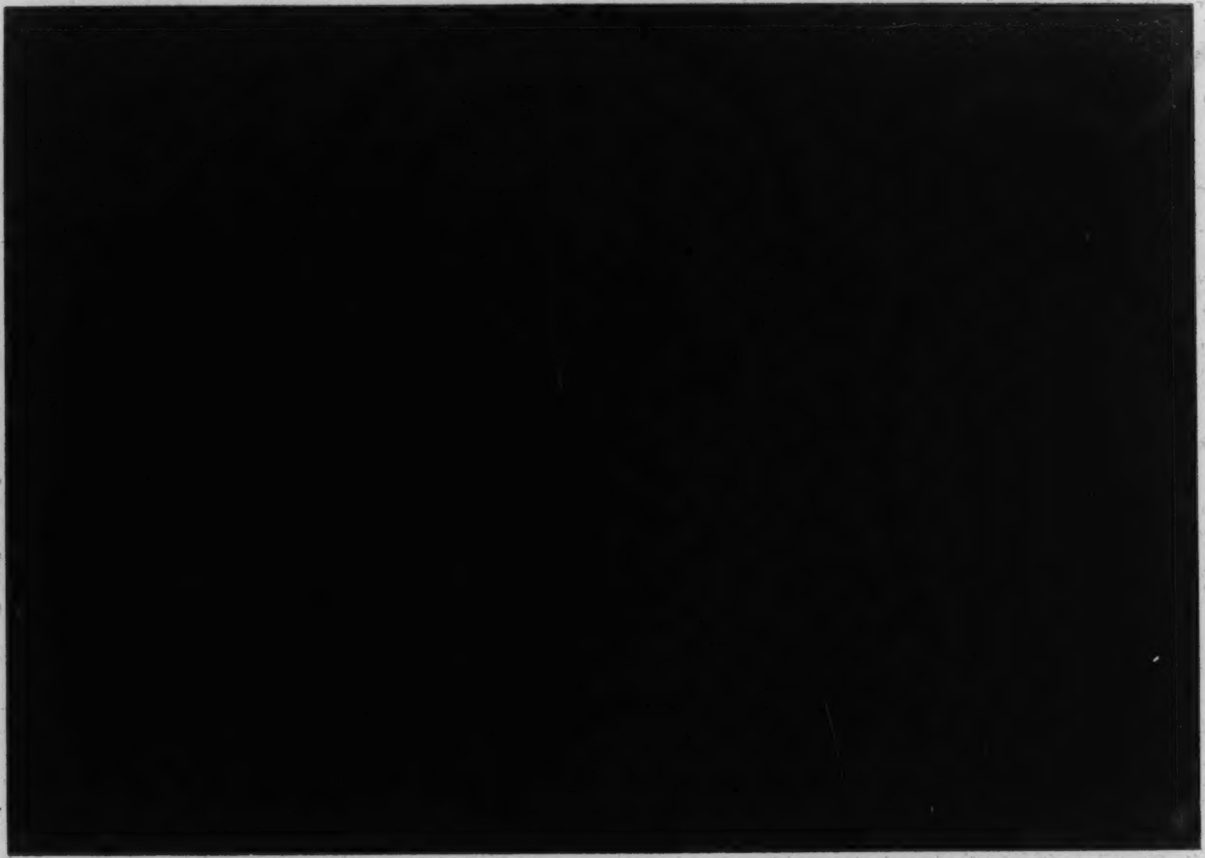


Figure 4.9b Predominant Cyanobacterial Species of Study Lakes *Anabaena flos-aquae* (Magnification 400x)

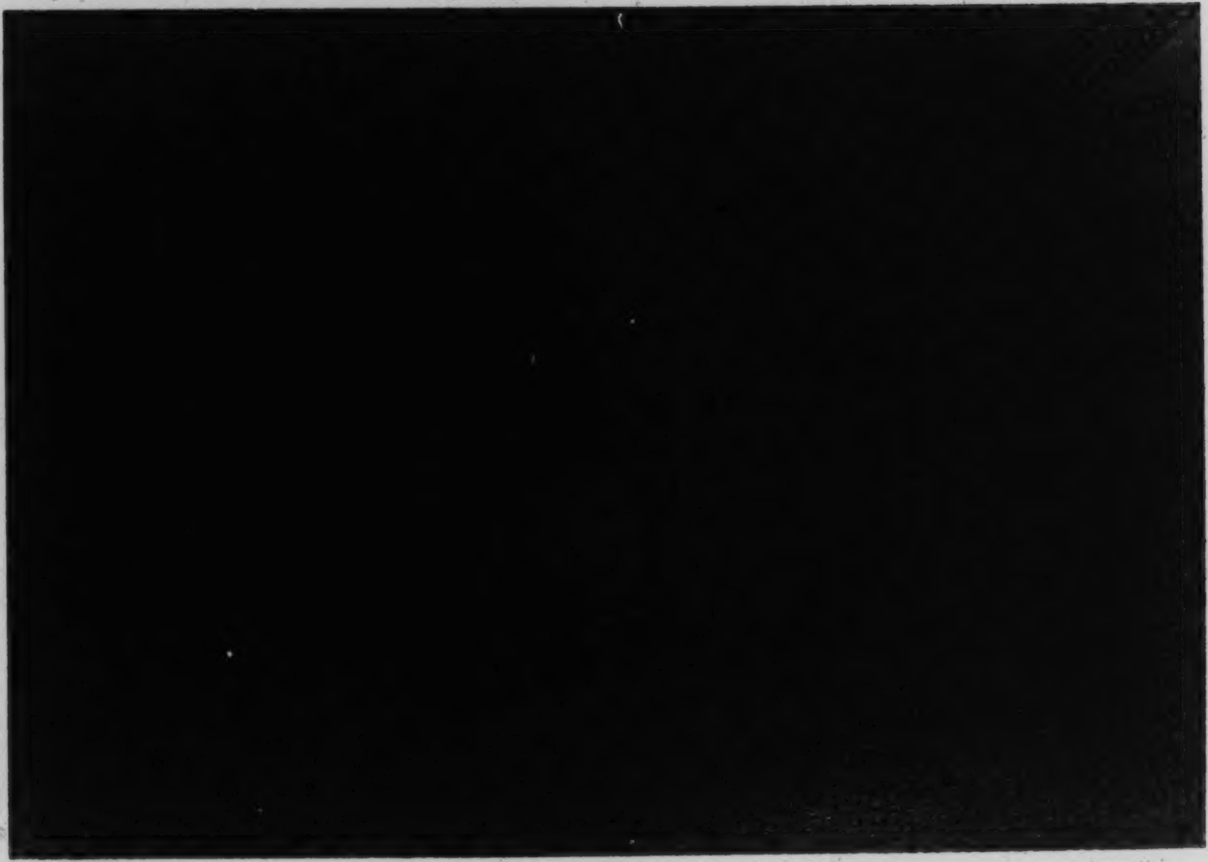


Figure 4.9c Predominant Cyanobacterial Species of Study Lakes *Aphanizomenon flos-aquae* (Magnification 400x)

4.4 Evaluation

4.4.1 Bioassays

Mouse bioassays were performed by Brian Kotak using facilities at the Saskatchewan Research Council. Approximately 50 mg of freeze dried algal biomass was suspended in 1600-µL 0.9% saline solution. The suspension was then sonicated to lyse the cells and allow release of the toxin to the solution. The samples were centrifuged and the supernatant was collected for bioassay. Supernatants were injected intraperitoneally into male Swiss SRV mice at a dose of 20-µL supernatant per gram of mouse body weight.

The liver of each mouse was weighed immediately after death (mice which had not died within 24-h were sacrificed) and visible signs of liver damage were noted. Results from these experiments were used only for confirmation of acute toxicity of samples shown to contain toxins. Details of these experiments will be reported by Brian Kotak.

4.4.2 Hepatotoxin Isolation

Microcystis aeruginosa cells collected from Coal Lake on Aug. 10, Aug. 16, Aug. 22, Aug. 26 and Aug 30 were combined after freeze-drying to yield a total biomass of 6.5 g. The toxin isolation procedure was a modification of that of Harada et al. (1988). The freeze-dried cells were extracted twice with 5% acetic acid for 1-h with stirring. The extract was centrifuged at 10,000 g for 30-min and the supernatant was applied to a disposable C18 cartridge. The cartridge was washed with 10% methanol, the toxic fraction was eluted with methanol and the solution was evaporated to dryness. The residue was subjected to flash chromatography on silica (Kieselgel 60, 230-400 mesh) using the lower layer of a chloroform : methanol : water (65:35:10) solvent system.

Fractions which were monitored on silica gel TLC plates (Kieselgel $60F_{254}$), using a chloroform : methanol (1:1) development system and pure microcystin LR standard as the reference. Fractions containing microcystin LR were evaporated to near dryness using a rotary evaporator and the residue was subjected to preparative TLC on silica (Kieselgel $60F_{254}$). The plate was developed in a pre-equilibrated TLC chamber using chloroform : methanol (1:1). The developed plate was viewed under short wavelength UV light and the fluorescent band corresponding to the microcystin LR standard spot was marked. Silica gel on this area of the plate was scraped from the plate and washed with solvent to dissolve the microcystin LR. Removal of the solvent gave 4 mg of the compound.

4.4.3 Batch Toxin Release

Concentrated samples of algal cells were collected on three separate dates from a natural bloom in Coal Lake (Figure 4.10). The bloom material was collected on August 26, 1990, September 13, 1990 and September 23, 1990 with a plankton tow net. Bloom material was then mixed with a volume of lake water and transported directly to the laboratory. Water bloom material corresponding to 10 - 15 g dry weight of *Microcystis aeruginosa* were placed in 15-L borosilicate glass containers together with lake water obtained at the same sampling point to give a total volume of 10.7 L. A magnetic stirrer was used to homogenize the mixture and 100-ml homogeneous samples were withdrawn through a tap at the bottom of each container to determine

Figure 4.10 Algal Bloom at Coal Lake August 1990

the mass of algal cells present in each vessel. Three containers of bloom material were studied in each trial (Figure 4.11). One was used as a control, one was treated with copper sulfate and one was treated with calcium hydroxide (lime).

The experiment was performed with reference to a procedure established by Berg et al. (1987) and was carried out in a temperature controlled room with the thermostat set at 20 °C ± 2 °C. The room was illuminated with fluorescent lighting (10 x General Electric cool white F40CW) in a 12 - 12 h light-dark cycle. The illumination near the containers during the experiment was measured with a Protomatic photometer and was found to be approximately 900 lux at the surface of the containers.

After 12 hours, the bloom material had separated into two distinct phases of concentrated algal cells floating on the top of almost clear lake water. The bloom material was then treated with the chosen bloom control chemicals. One container was treated with 2 mg/L $CuSO_4$ (500 µg/L Cu) applied with an aerosol sprayer evenly over the floating algal cells. Another container was treated with 100 mg/L $Ca(OH)_2$ with a second aerosol sprayer evenly over the floating algal cells. The third container was adjusted to an equal volume through the addition of distilled water.

Samples were collected after a specific time period from each container. Samples for trial #1 were collected through the tap at the bottom of each container and samples for trials #2 and #3 were collected with tygon tubing connected to a small liquid pump operating at a flow rate of 100 mL/minute. Samples removed with the pumping system were withdrawn at all depths to approximate a homogeneous sample of the water phase and samples were then analysed as described above.

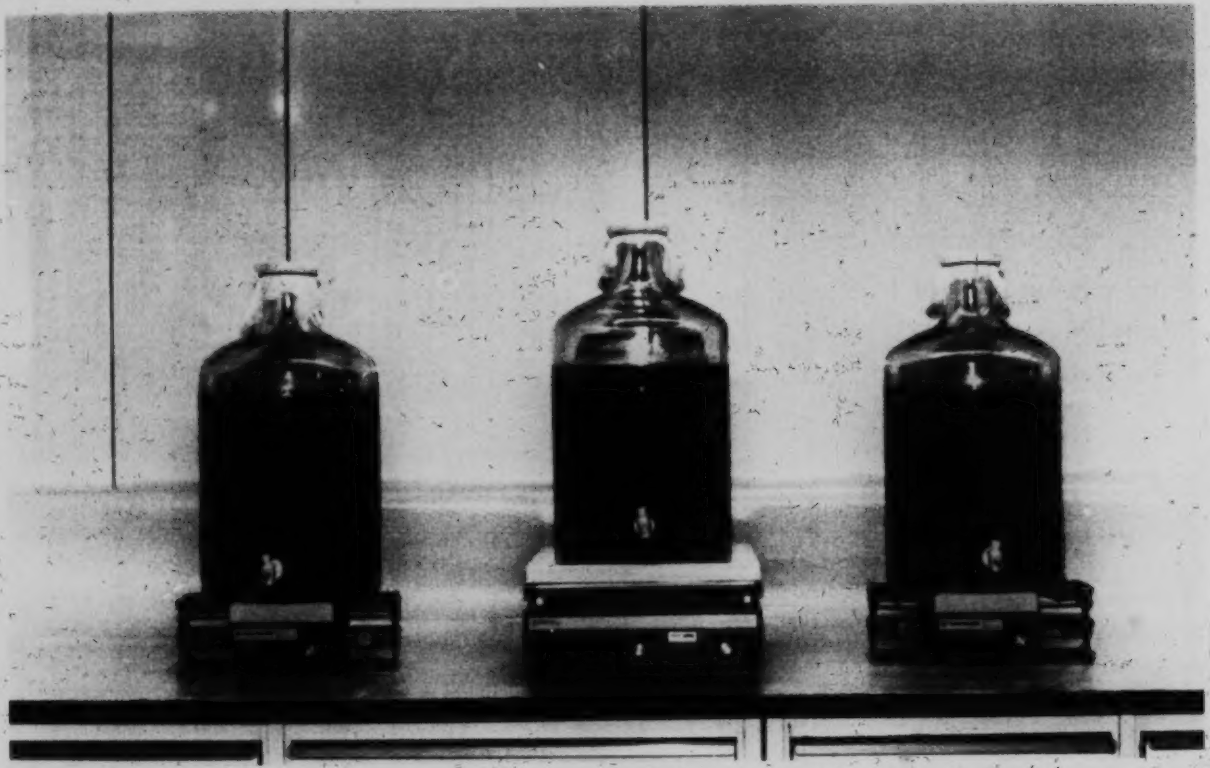


Figure 4.11 Batch Toxin Release Apparatus

5. RESULTS AND DISCUSSION

5.1 Batch Toxin Release Studies

Batch toxin release studies were dependent upon being able to collect large quantities of biomass which could be quickly transported to the laboratory and subjected to experimentation. Three batch trials were carried out sequentially over a period of 4 weeks during which the health of the natural blooms was degrading. The batch toxin release studies were carried out at a constant temperature of 20°C and under continuous 12-h light-dark cycles. Prior to treatment with lime and copper sulfate, the algal cells were allowed to separate out to form a thick layer at the top of each vessel (Figure 5.1a). This had generally taken place after leaving the cells overnight. The biomasses were higher than would be expected in a natural bloom, but the levels used had to be selected to ensure detectable toxin levels in both the cells and the water phase for the duration of the experiment.

Because the biomasses were higher than natural situations, copper sulfate doses of 2 mg/L (500 μg/L Cu) and lime doses of 100 mg/L were used. The copper sulfate was added to one vessel and the lime was added to another vessel, leaving one container with untreated cells to be used as a control. After a period of 24-h there was a very definite visible change in the two treated batches. A large number of cells settled to the bottom of the container which had been treated with lime, although there was no noticeable colour change in the cells. The vessel containing the cells treated with copper sulfate showed a distinct, deep blue-green colouring of the water phase (Figure 5.1b). A copper sulfate solution of 2 mg/L (500 μg/L Cu) is dilute and does not have a noticeable blue colour, thus this release of pigment indicates that the cells treated with copper sulfate have been lysed and the internal

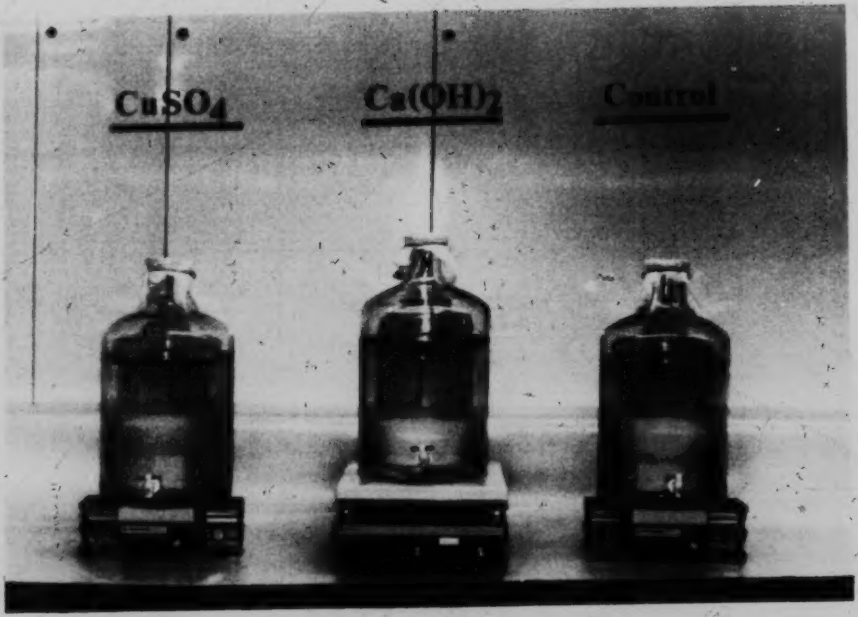

CuSO$_4$
Ca(OH)$_2$
Control

Figure 5.1a   Batch Microcystin LR Release (Time 0)

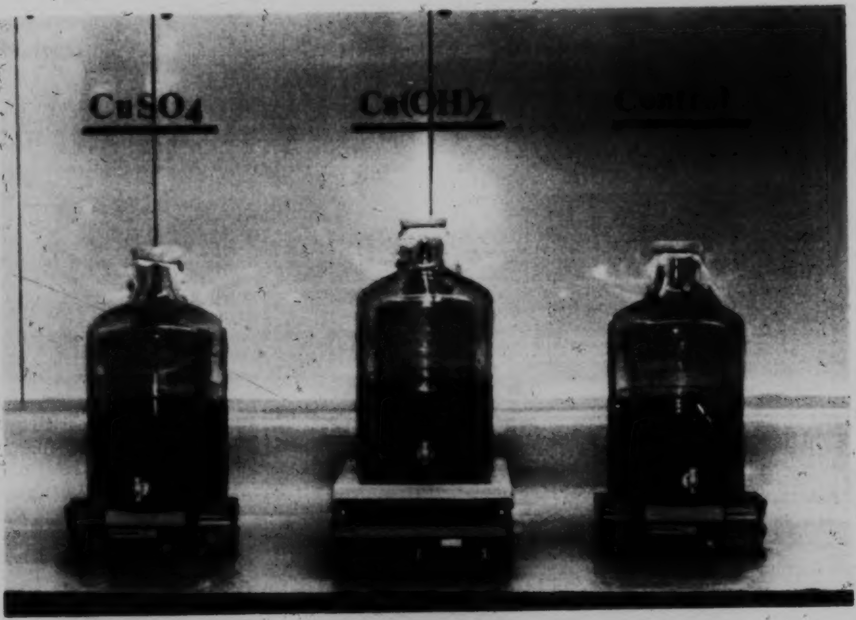

CuSO$_4$
Ca(OH)$_2$
Control

Figure 5.1b   Batch Microcystin LR Release (Time 1 Day)

contents of the cells have been allowed to escape to the water phase. This did not appear to be the case in either the cells treated with lime or the untreated cells.

The amount of microcystin LR released to the water phase for each group of cells was monitored at specific times for each trial. The measured amounts of toxin released to the water phase relative to time of sampling for each trial are shown in Figures 5.2 to 5.4. All measured data are summarized in the appendix. During the first trial, 4 samples were taken in the first day but it became obvious that there were very few cells lysed during this time period. The next two trials were sampled daily. All three trials demonstrated a rapid release of toxin to the water phase after treatment with copper sulfate, with a maximum toxin release after 2 to 4 days. After the period of maximum toxin release there appeared to be a decomposition of the toxin taking place, because toxins levels steadily decreased until the end of the experiment in all three cases. The cells treated with lime showed very little toxin release in all three trials, indicating that although the cells settled to the bottom after lime treatment, they are not lysed in the process. The untreated cells did not release significant levels of toxin in trial #1 but did release small amounts in trial #2 and #3. This difference between trials was probably due to the condition of the cells being used. The cells used for trials #2 and #3 were beginning to collapse when first brought to the laboratory, and therefore may have been decomposing more rapidly during the tests. The apparent microcystin LR transfer rates from the untreated cells to the water phase vary from the results of Berg et al. (1987) where no toxin was released until day 26. These observations suggest that the toxin release likely depends on the status of the algal cells being studied.

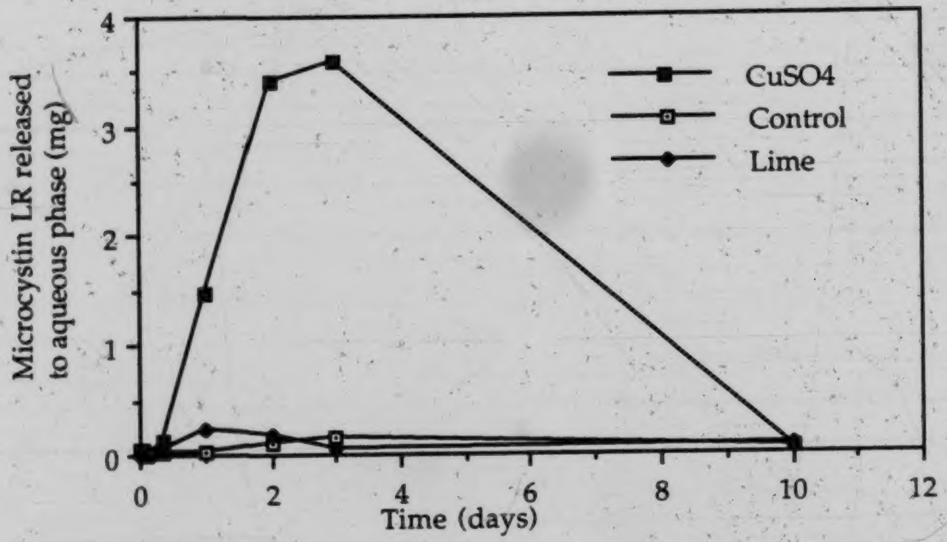


Figure 5.2 Batch Microcystin LR Release Trial #1

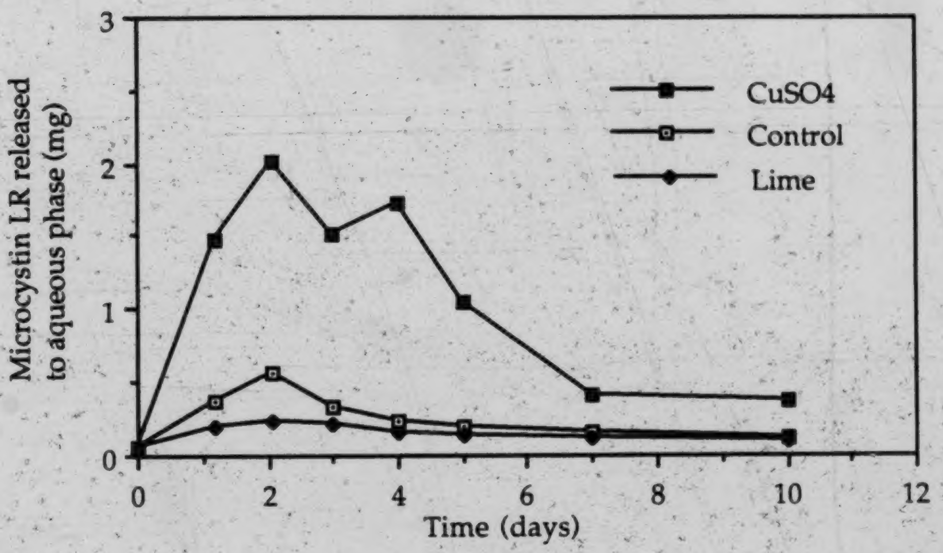


Figure 5.3 Batch Microcystin LR Release Trial #2

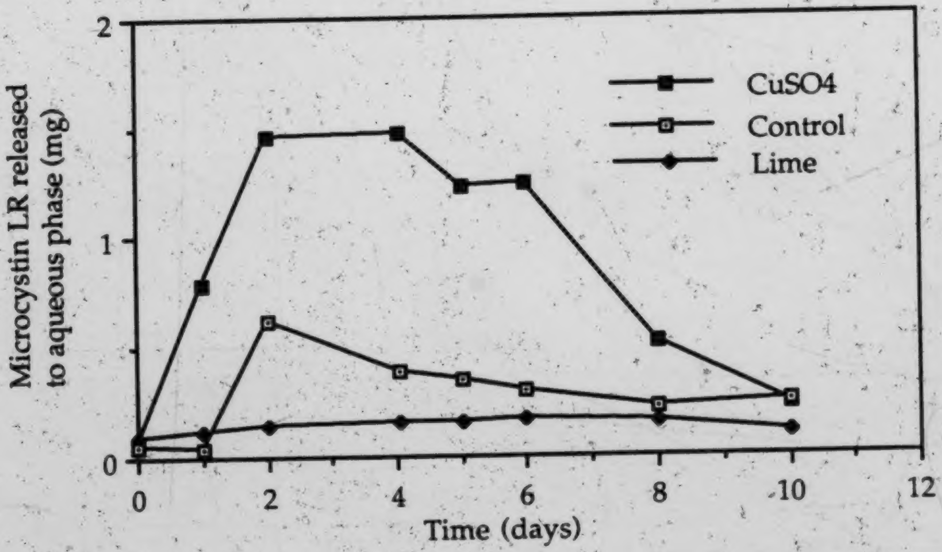


Figure 5.4 Batch Microcystin LR Release Trial #3

In order to investigate what was happening to the cells after treatment, toxin-producing *Microcystis aeruginosa* was grown by Dr. Hans Peterson of the Saskatchewan Research Council using a culture obtained from Dr. Wayne Carmichael. The algal cells were divided into three parts; one portion was treated with 75 mg/L $Ca(OH)_2$, one portion was treated with 10 µM $CuSO_4$ and one portion was left untreated. After 24-h, cells from each portion were removed with filtration through a 0.45-µm membrane filter and the collected cells were studied by scanning electron microscopy (SEM). Cells from each portion were also immobilized and sliced into 1-µm thick sections to allow for study by transmission electron microscopy (TEM). The micrographs of each type of cell are shown in Figures 5.5 to 5.7.

The microscopic representations clearly show what was happening to the cells in each case. The SEM photographs indicate that the control cells and the lime treated cells are in similar condition, while the copper sulfate treated cells appear to have collapsed. The TEM photographs clearly indicate that the membrane of the control cell was still intact, as was the membrane of the lime treated cell, although there was an obvious coating around the lime treated cells. The membranes of the cells treated with copper sulfate are no longer intact and the overall integrity of the cells has been lost. These representations indicate toxins should be released rapidly to the water phase after treatment with copper sulfate but not after treatment with lime. Thus they confirm the observations of the three batch trials.

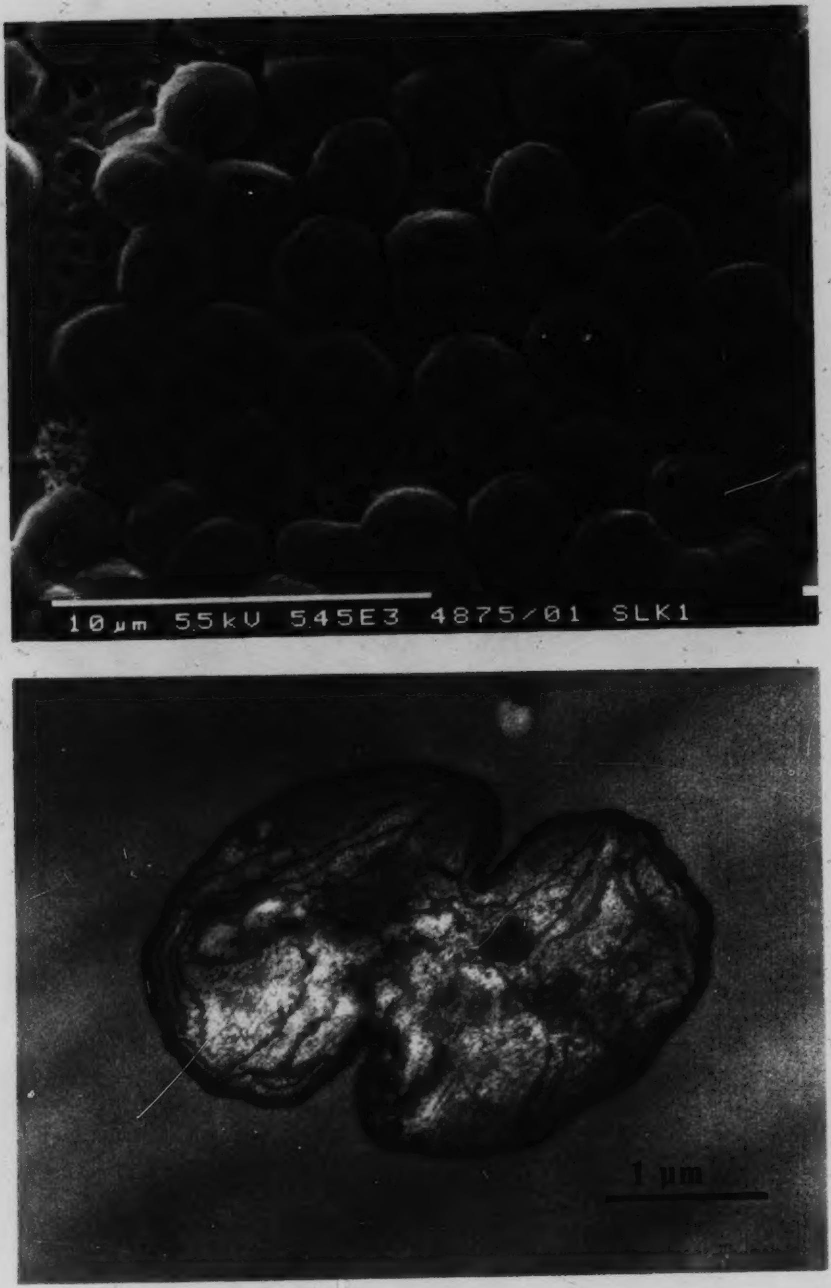

10 µm 55kV 545E3 4875/01 SLK1
1 µm

Figure 5.5 Untreated *Microcystis aeruginosa* cells.
Top: Scanning Electron Microscopy view.
Bottom: Transmission Electron Microscopy view.

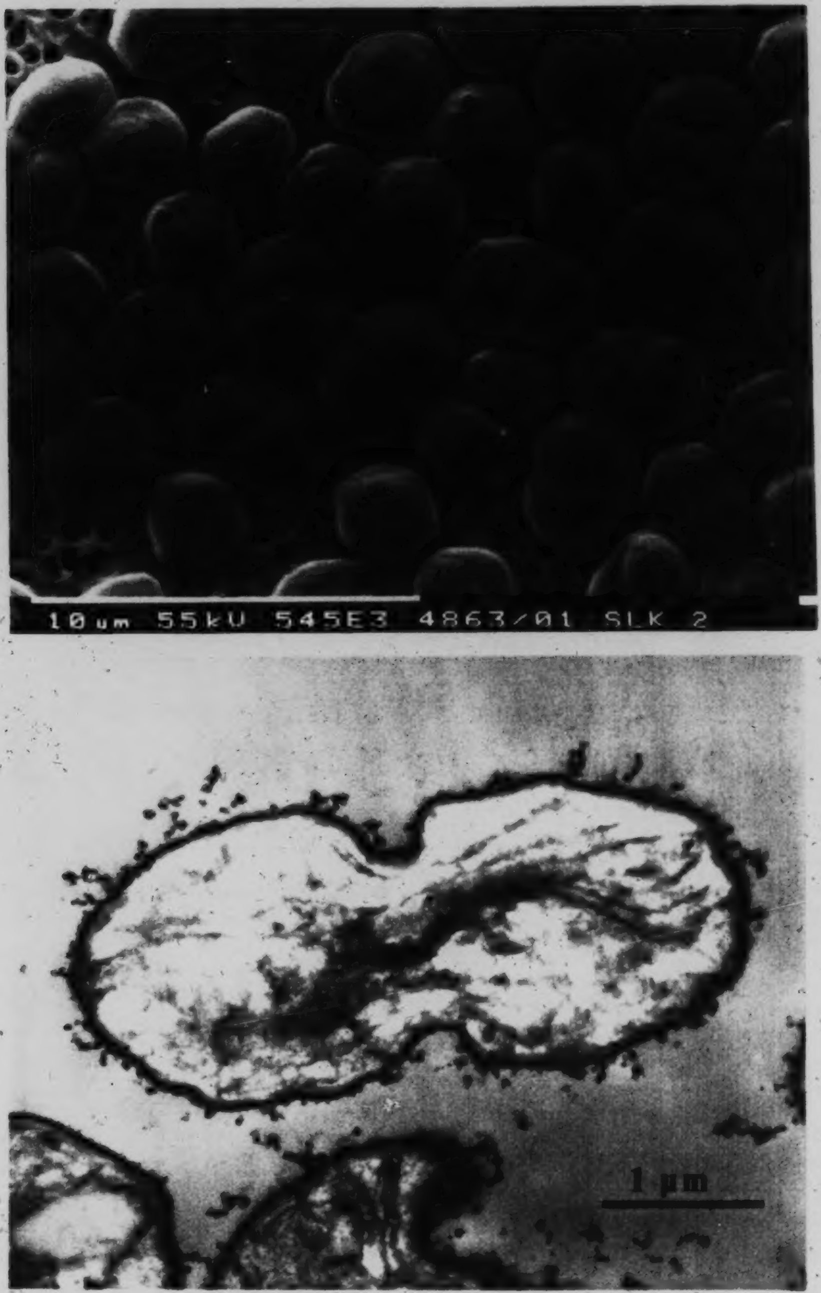

10 um 55kU 545E3 4863/01 SLK 2
1 µm

Figure 5.6 *Microcystis aeruginosa* cells treated with 75 mg/L $Ca(OH)_2$
Top: Scanning Electron Microscopy view.
Bottom: Transmission Electron Microscopy view.

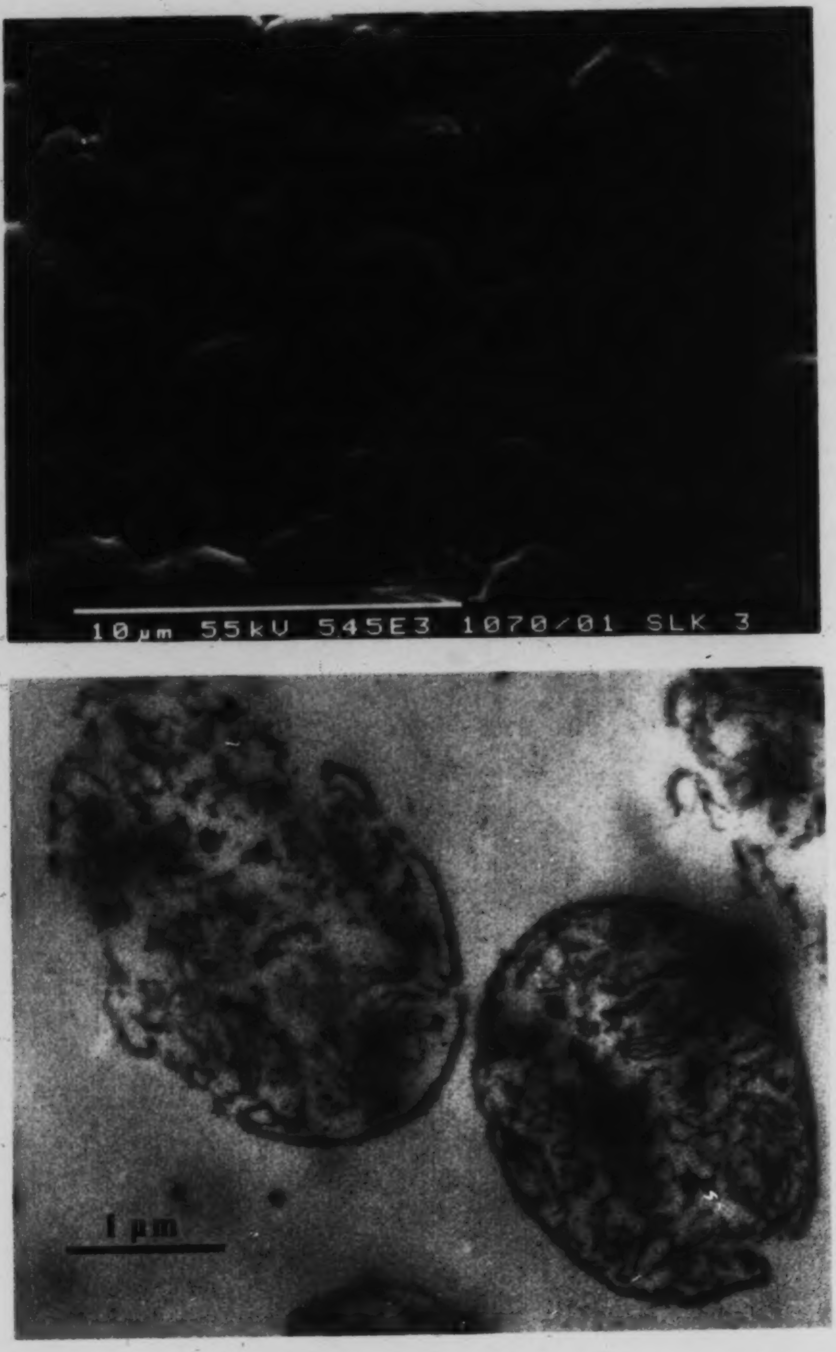

10µm 55kV 545E3 1070/01 SLK 3
1 µm

Figure 5.7 *Microcystis aeruginosa* cells treated with 0.010 mM $CuSO_4$
Top: Scanning Electron Microscopy view.
Bottom: Transmission Electron Microscopy view.

5.1.1 Toxin Distribution

It was not possible to measure the total amount of toxin present in each vessel prior to the batch tests because such measurement is a destructive analysis. The initial amounts of toxin had to be estimated by analysis of an aliquot of the biomass. Because of this it was also impossible to calculate the exact distribution of toxin between the algal cells and the water phase. Graphical interpretation of the measured data was used to approximate the toxin distribution in the biological system being studied. The degradation of the toxins released to the water phase was assumed to be exponential (like other biological systems of decay).

The maximum amount of toxin which could be found in the water phase was approximated using the copper sulfate batch study toxin release data in Figures 5.8a and 5.8b. The aqueous toxin levels from the copper sulfate batch studies #2 and #3 were analysed, but the results of the first copper sulfate batch test could not be used as the sampling intervals used were not adequate to monitor the toxin decay. An analysis of an initial aliquot of the biomass in the copper sulfate batch study #2 and #3 gave estimated total toxin concentrations in the vessels of 1.9 mg and 1.8 mg respectively. These values were used to approximate the C_0 for a first-order exponential decay curve of the form

$$C = C_0 \exp^{(-kt')} \qquad (1)$$

The estimated time of maximum concentration (C_0) was then used as $t'=0$, and the decay coefficient (k) was calculated. The decay coefficient was found to be 0.25 day^{-1} for both experiments. This calculated k value was then used to calculate $t_{1/2}$ for each trial. The calculated exponential decay half life from $t'=0$ (i.e. time at maximum toxin release) was 2.8 days in both experiments.

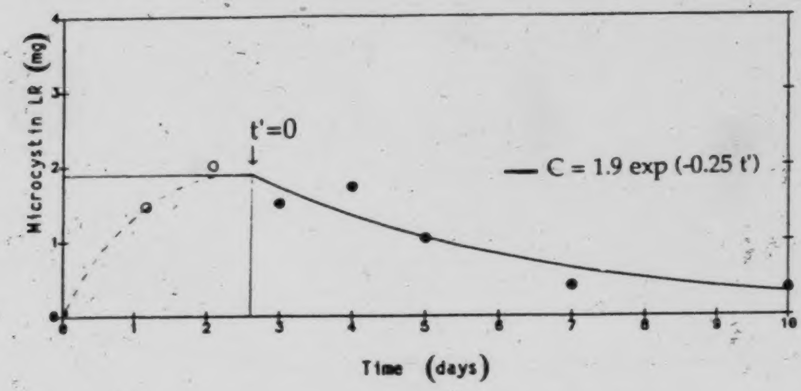


Figure 5.8a Interpretation of Batch Microcystin LR Release (Copper Sulfate) Trial #2

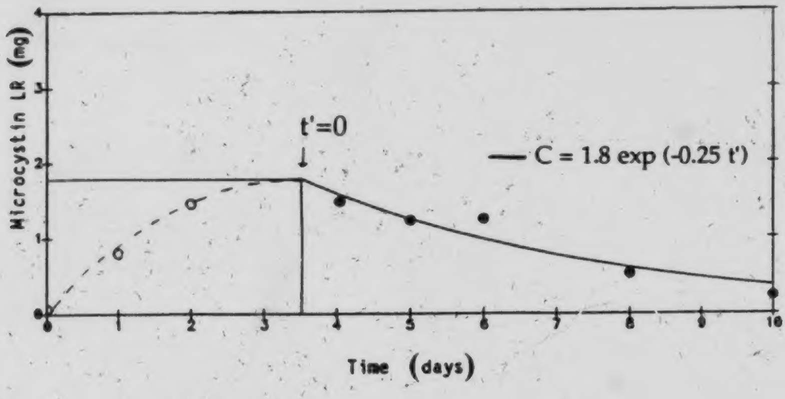


Figure 5.8b Interpretation of Batch Microcystin LR Release (Copper Sulfate) Trial #3

5.1.2 Analytical Toxin Recovery Evaluation

Two experimental variables were evaluated for their effects on the levels of toxins released during the experiments. The first variable tested was the method of lysing of the cells. Lysing of the cells with $CuSO_4$ instead of lysing during the freeze-drying process could result in significantly different measured microcystin LR levels. The effect of time was the second variable of the batch studies to be tested. It is possible that a significantly different mass of microcystin LR was released as the cells lysed naturally over time.

In the first experiment, 4 separate cultures of cells containing microcystin LR were split in half and one half was treated with 2 mg/L $CuSO_4$ and left for 48 h. All samples were then freeze-dried, extracted and analysed for the hepatotoxin. A second group of three natural samples of algal cells known to contain microcystin LR were extracted for both 1 day and for 3 days prior to analysis. Results of these tests are summarized in Tables 5.1 and 5.2.

The t-test for paired data was used to determine if there was a significant difference between each pair of treatments. This test was used because each set of data was obtained using the same algal sample subjected to two different treatments. When applying the paired t-test, the mean difference between the two sets of data was considered as the variate and it was compared with its standard deviation. This removed the test-to-test variation, although there was a corresponding loss in precision because the standard deviation was based on fewer degrees of freedom (Kennedy and Neville 1976). The results summarized in Table 5.3 demonstrate that there was no significant difference in measured toxin levels at the 95% confidence level for either set of samples. Neither the time of extraction nor the lysing of cells with $CuSO_4$ caused a significant variation in the amount of toxin measured in the biomass.

Table 5.1 Toxin Recovery After Copper Sulphate Treatment

Sample	Culture Date	Control Micrcystin LR µg/g Biomass	CuSO4 Microcystin LR µg/g Biomass
Coal Lake	14-Dec-90	30	26
Pretzlaff	14-Dec-90	111	107
45-5A	7-Dec-90	102	119
45-5A	4-Dec-90	114	119

Table 5.2 Toxin Recovery Based on Time of Extraction

Sample	Culture Date	1 Day Extraction Microcystin LR µg/g Biomass	3 Day Extraction Microcystin LR µg/g Biomass
Coal Lake	26-Aug-90	396	400
Coal Lake	13-Sep-90	136	143
Coal Lake	24-Sep-90	119	122

Table 5.3 Paired t-test Values for Toxin Recovery Studies

Comparison	d.f.	Mean Difference	Paired t-value	*P* (t)
Control & CuSO4	3	3.50	0.7*	*P* >.55
1 day & 3 day extraction	2	4.67	3.88*	*P* >.08

* No significant difference at 95% confidence level

5.2 Evaluation of Raw Water Supplies

The lakes and dugouts which were studied for this research were chosen because they serve as raw water supplies for municipal water treatment plants or for the domestic water treatment systems used by rural households. Consequently the occurrence of cyanobacterial toxins in these sources would be relevant to human health.

Climatic conditions during the summer of 1990 were obtained (Environment Canada 1990) and water characteristics were monitored, along with algal biomass, cyanobacterial species present, and corresponding toxin levels measured in the algal cells. Raw and treated water samples from the three municipal water treatment plants which draw raw water from the study lakes were analysed for the presence of the hepatotoxin microcystin LR. Lake water samples were analysed for odorous compounds commonly found in waters containing cyanobacteria and algal cell toxin levels were compared to the presence or absence of these odorous metabolites.

A verification of the toxicity of the algal cells collected was also carried out through the use of a limited number of mouse bioassays.

5.2.1 Weather Trends and Temperatures

The weather in Alberta during the summer of 1990 was warm enough to favour the formation of cyanobacterial blooms in many parts of the province. In general cyanobacteria will grow rapidly and form a bloom if there is adequate light, little wind and a water temperature of at least 15 °C (Skulberg et al. 1984) These criteria were met in early June for many of the small dugouts and favourable conditions were observed at the study lakes when sampling began at the end of July.

The general trend in daily average temperature, windspeed and hours of bright sunshine in central Alberta is shown in Figure 5.9 (Environment Canada, 1990). The more specific conditions at Camrose and Wetaskiwin are shown in Figures 5.10 and 5.11 (Environment Canada, 1990; data obtained prior to publishing). All data are tabulated in the appendix. No specific meteorological conditions are monitored at the Village of Ferintosh but climatic conditions were very similar to those observed at Coal Lake and Driedmeat Lake. These figures indicate that there was a period of relative calm combined with long hours of bright sunshine during the latter half of July. These conditions, when combined with temperatures which were warm enough to heat the water to over 20 °C at the beginning of August, were favourable for the rapid growth of a number of cyanobacteria species.

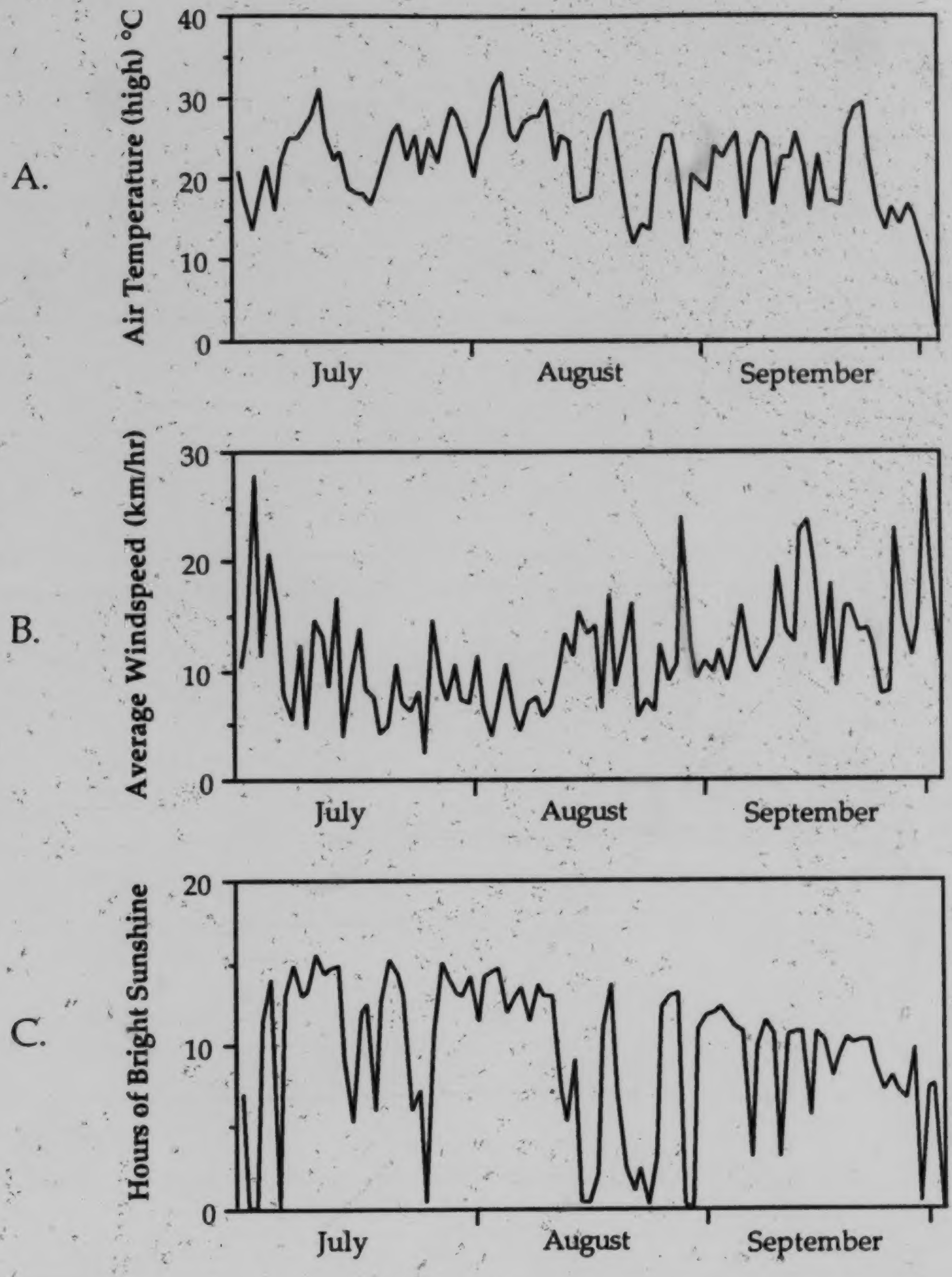


Figure 5.9 Central Alberta Weather (Edmonton International Airport)
A. Daily High Air Temperature
B. Daily Average Windspeed
C. Daily Average Hours of Bright Sunshine
(adapted from: Environment Canada 1990)

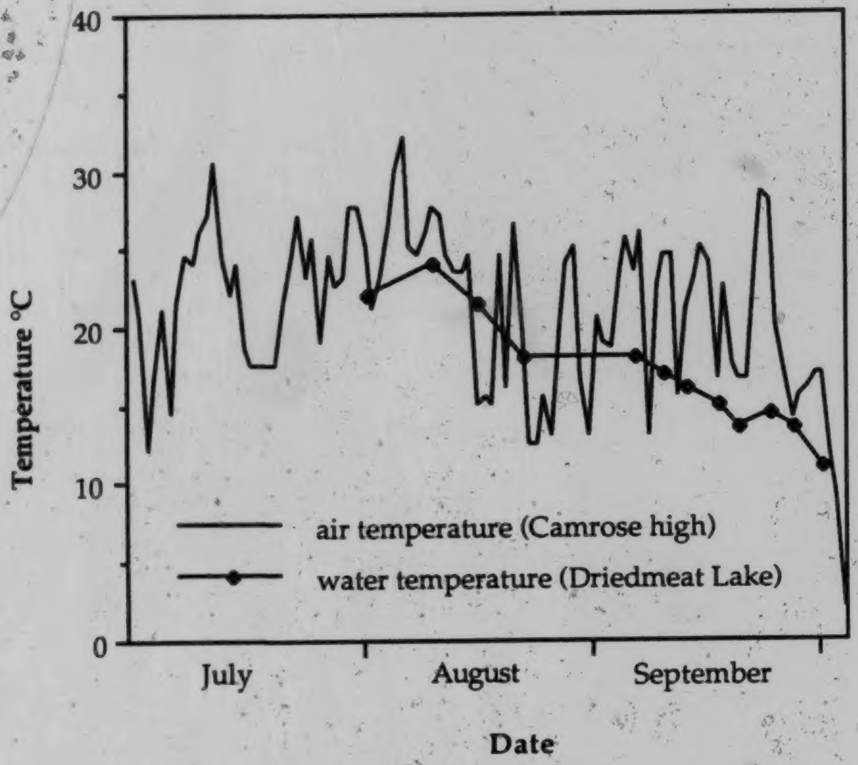


Figure 5.10 Daily High Air Temperature at Camrose (adapted from: Environment Canada 1990) and Surface Water Temperature in Driedmeat Lake

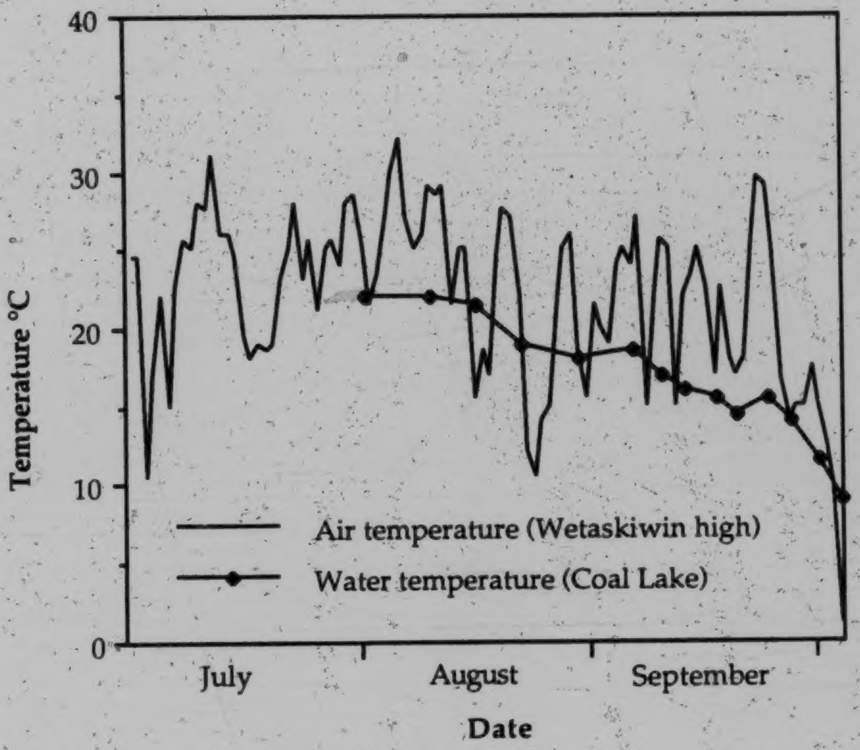


Figure 5.11 Daily High Air Temperature at Wetaskiwin (adapted from: Environment Canada 1990) and Surface Water Temperature in Coal Lake

5.2.2 Algal Species Distribution

Algal samples, collected from each lake or dugout, were found to contain a number of algal species with distributions varying continuously over the summer. Expected species of cyanobacteria such as *Microcystis aeruginosa, Anabaena flos-aquae* and *Aphanizomenon flos-aquae* were found in many of the samples, along with a number of other blue-green algal species. Algal species distribution for the lake samples collected are summarized in Figures 5.12, 5.13 and 5.14. Percentages of each species are approximate and are included only to illustrate the general trends in species domination. These figures demonstrate the dynamic nature of the distribution of the various species of cyanobacteria. Only Little Beaver Lake was dominated by one cyanobacterial species throughout the sampling period. This may indicate that the *Aphanizomenon flos-aquae* species prefers conditions which are slightly different than those preferred by *Microcystis* or *Anabaena* and these conditions were present in the small and shallow Little Beaver Lake throughout the summer.

Microcystis aeruginosa cells were found in all three study lakes but were only found in one dugout (Pretzlaff). All samples which were found to contain *Microcystis* cells were extracted and analysed for microcystin toxins. *Anabaena flos-aquae* cells were found in Driedmeat Lake along with one Helbig dugout, the Pretzlaff dugout and three Peace River area dugouts. All samples which were found to contain *Anabaena* cells were extracted and analysed for both microcystin toxins and anatoxin-a.

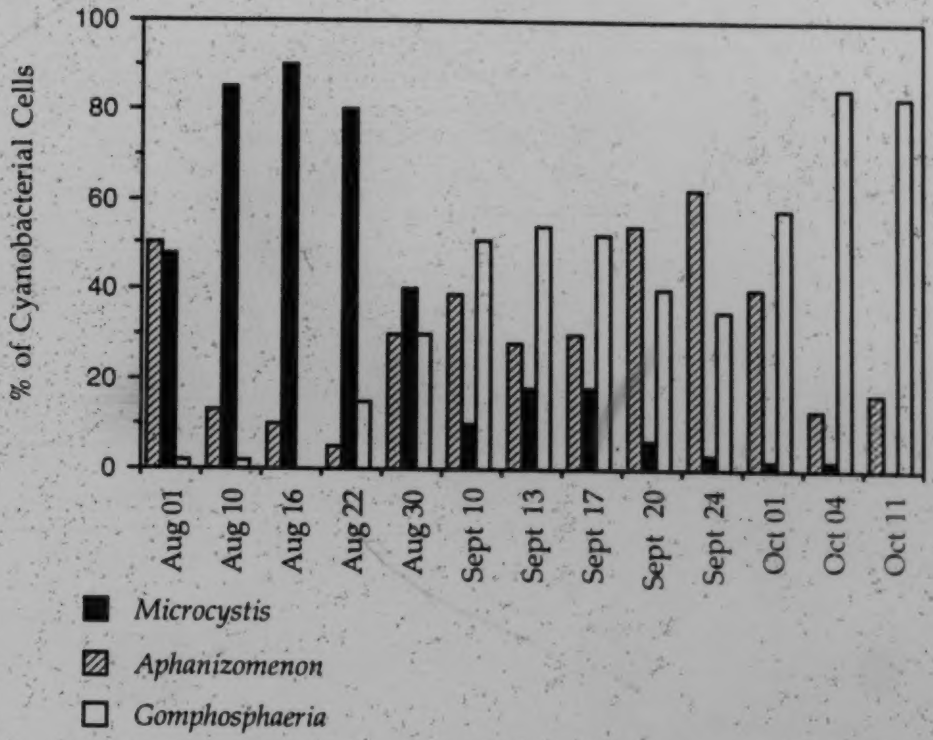


Figure 5.12 Cyanobacterial Cell Distribution of Coal Lake

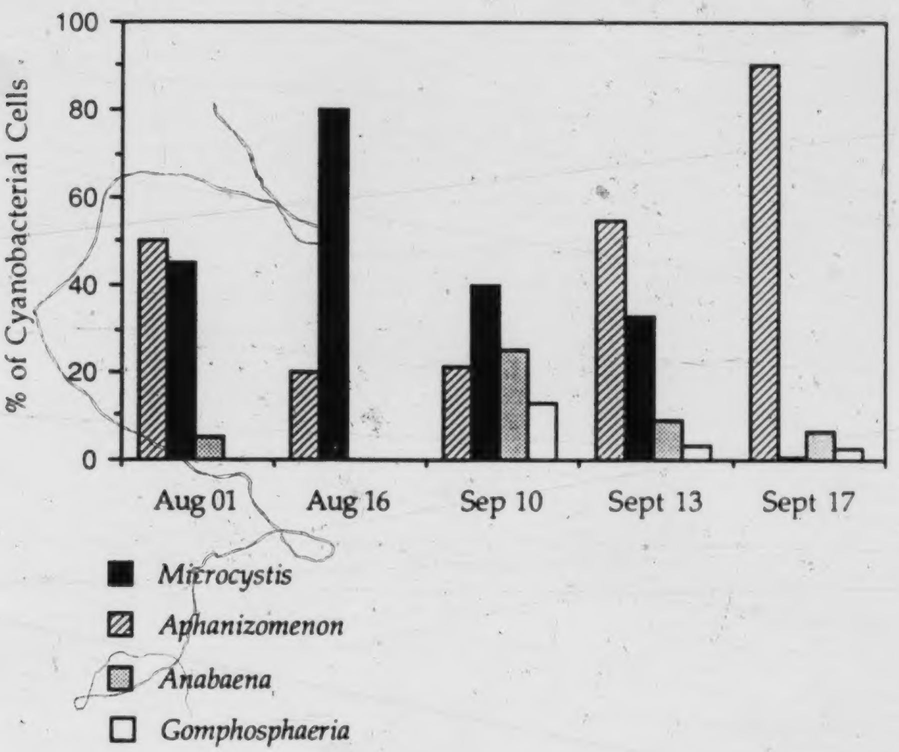


Figure 5.13 Cyanobacterial Cell Distribution of Driedmeat Lake

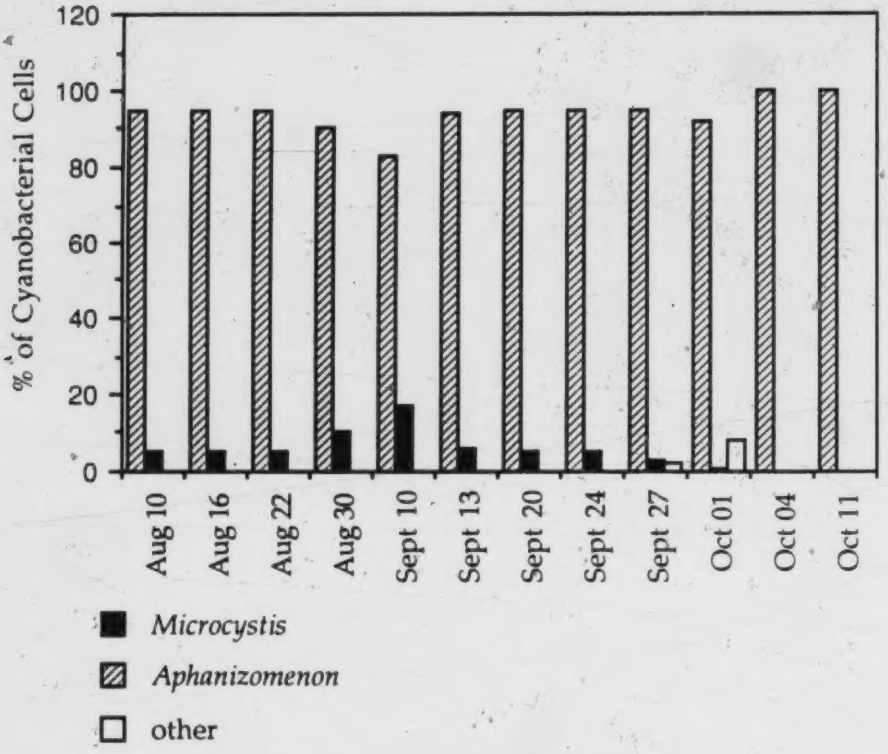


Figure 5.14 Cyanobacterial Cell Distribution of Little Beaver Lake

5.2.3 Hepatotoxin

The algal samples which contained either *Microcystis aeruginosa* or *Anabaena flos-aquae* were extracted and analysed for the presence of microcystin LR and microcystin RR, the two cyanobacterial hepatotoxins which were available as standards in our laboratory. None of the samples contained detectable amounts of microcystin RR and only the samples which contained *Microcystis* cells contained detectable amounts of microcystin LR. The hepatotoxin was detected in all three of the study lakes, but was not detected in any of the agricultural dugouts. Measured LR concentrations were reported on a mass of toxin per mass of dry biomass weight basis. This biomass included all species of algae present as well as zooplankton which were not filtered out prior to lyophilization of the algal cells. Algal species distributions and corresponding microcystin LR levels are listed in the appendix. The relative distribution of *Microcystis* spp. and microcystin LR extracted from the biomass is portrayed in Figures 5.15 to 5.17 for Coal Lake, Driedmeat Lake and Little Beaver Lake.

The data which were obtained for the Coal Lake samples, shown in Figure 5.15, indicate that there was a general correlation between the growth of *Microcystis* spp. cells and the production of microcystin LR within the cells. The data collected for both Driedmeat Lake and Little Beaver Lake, shown in Figures 5.16 and 5.17, demonstrate an apparent time lag between the growth of Microcystis cells and the production of microcystin LR.

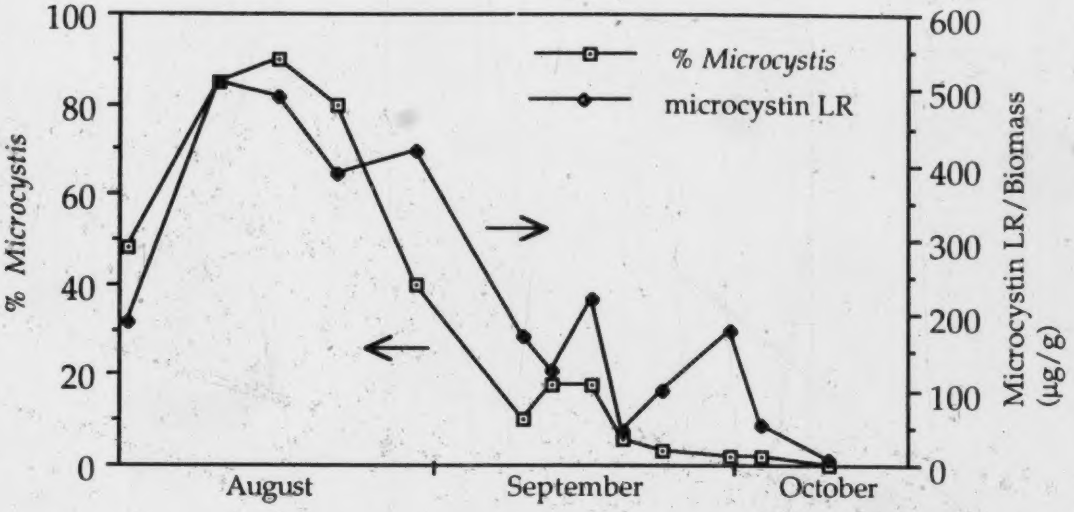


Figure 5.15 Relative Distribution of *Microcystis* spp. and Microcystin LR for Coal Lake

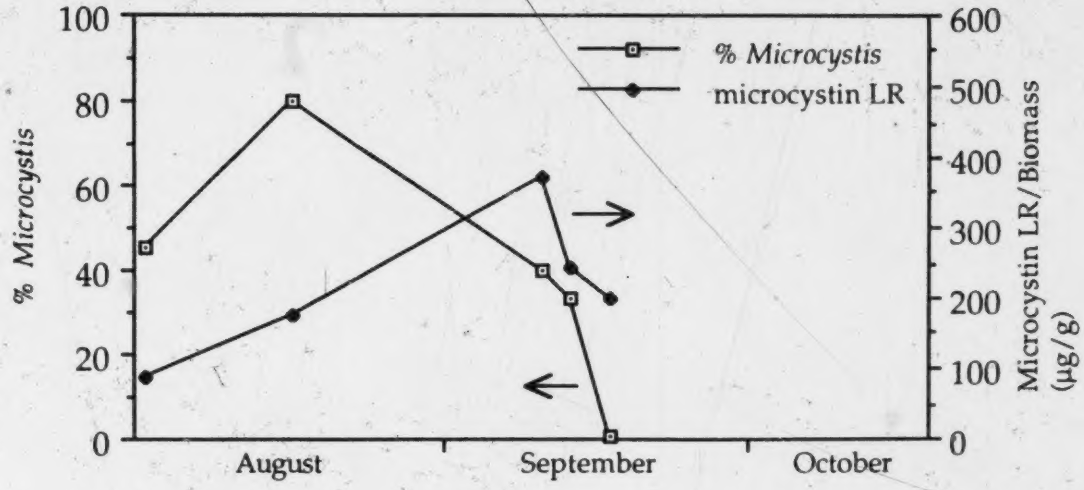


Figure 5.16 Relative Distribution of *Microcystis* spp. and Microcystin LR for Driedmeat Lake

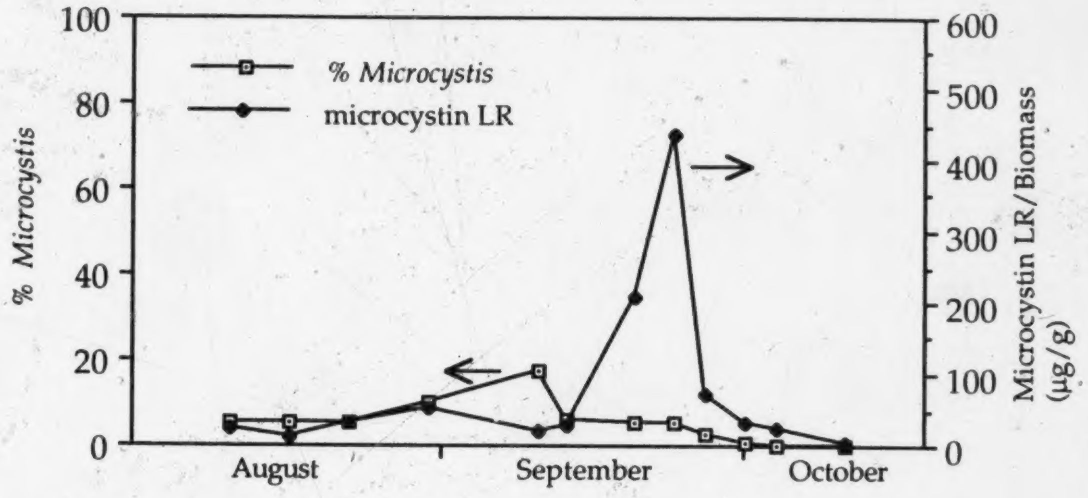


Figure 5.17 Relative Distribution of *Microcystis* spp. and Microcystin LR for Little Beaver Lake

5.1.3.1 Algal Biomass

The algal biomass in each natural water body can be estimated from measured chlorophyll α values. A general relationship is given in Standard Methods for the Examination of Water and Wastewater (APHA, AWWA and WPCF, 1989). The assumption is made that chlorophyll α constitutes, on the average, 1.5% of the dry weight of organic matter (ash free weight) of algae. Consequently algal biomass is calculated by multiplying chlorophyll α content by 67. The trend in chlorophyll α concentrations for each of the lakes sampled is shown in Figures 5.18 to 5.20. The average chlorophyll α level for Driedmeat Lake was 11.6 μg/L and this is typical of moderately productive lakes during occasional algal blooms. Coal Lake had an average chlorophyll α level of 29.9 μg/L and Little Beaver Lake had an average level of 29.1 μg/L which are levels typically found in eutrophic lakes which are well nourished and have algal blooms most of the summer (Mitchell and Prepas 1990).

General trends of total and dissolved phosphorus and inorganic nitrogen in the lake waters are also shown in Figures 5.18 to 5.20. It was also difficult to correlate the measured dissolved or total phosphorus to cyanobacteria growth or toxin production without data prior to the algal blooms. The phosphorus data do indicate total phosphorus concentrations typical of eutrophic freshwater lakes (Prepas and Trew 1983).

It is known that in general, low concentrations of available nitrogen will favour the growth of cyanobacteria which fix atmospheric nitrogen (Mitchell and Prepas 1990). Nitrogen levels measured in the three lakes generally follow this trend. Species distributions shown in Figures 5.12 to 5.14 indicate that percentages of *Microcystis* spp. (which cannot fix nitrogen) increase after nitrogen levels increase. In general species such as *Aphanizomenon* and *Anabaena*, which can fix nitrogen, predominate as

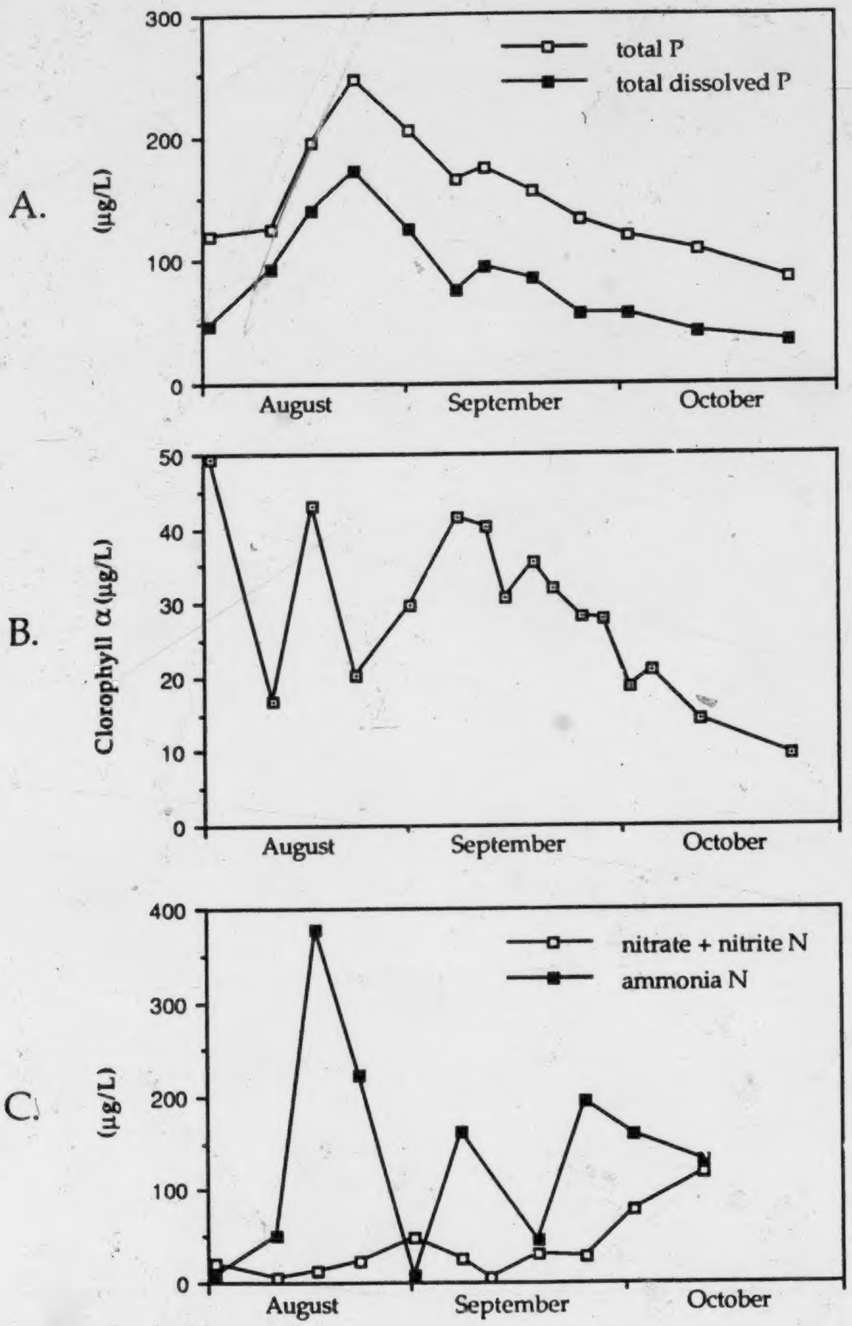


Figure 5.18 Coal Lake
A. Total Phosphorus, Total Dissolved Phosphorus
B. Chlorophyll α Concentrations
C. Nitrate + Nitrite and Ammonia N

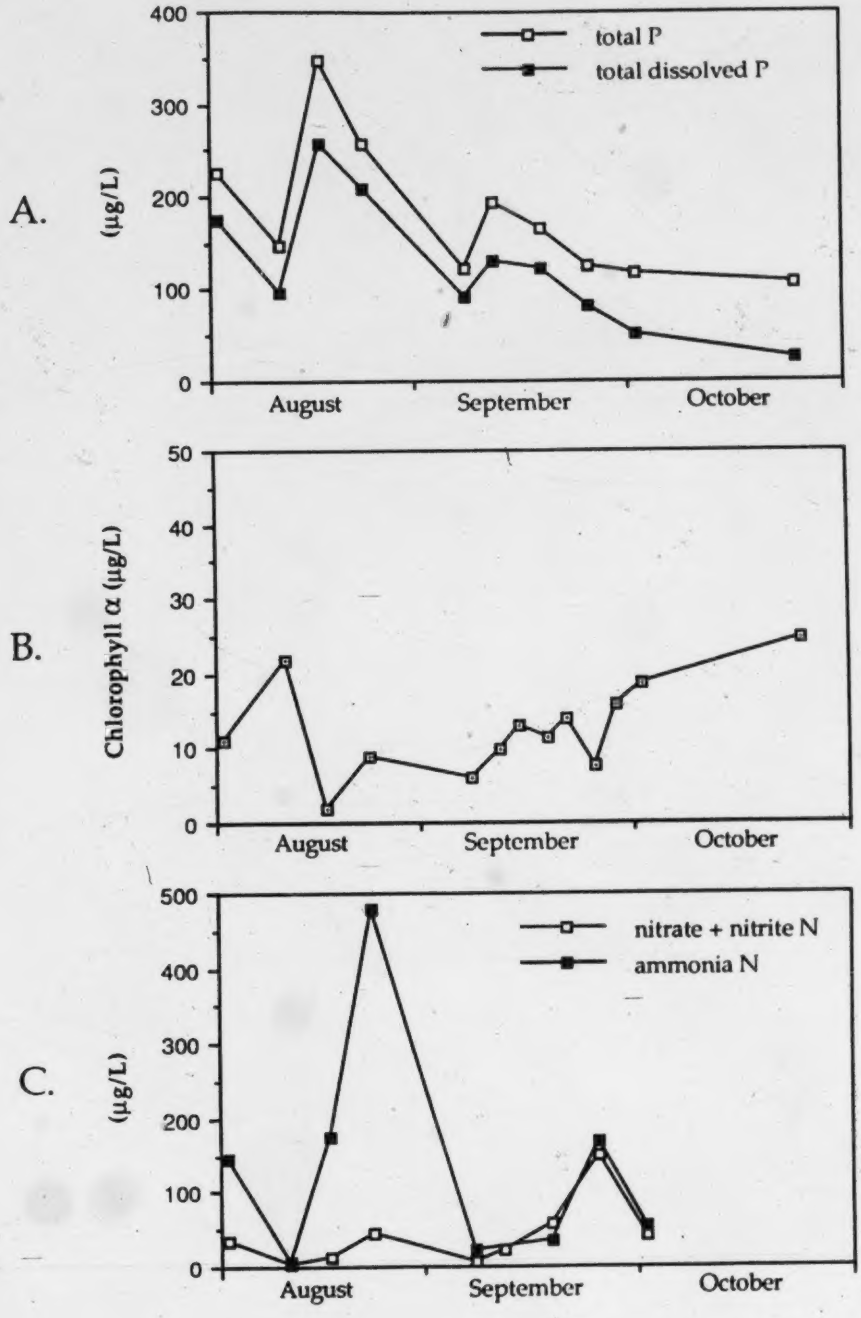


Figure 5.19 Driedmeat Lake
A. Total Phosphorus, Total Dissolved Phosphorus
B. Chlorophyll α Concentrations
C. Nitrate + Nitrite and Ammonia N

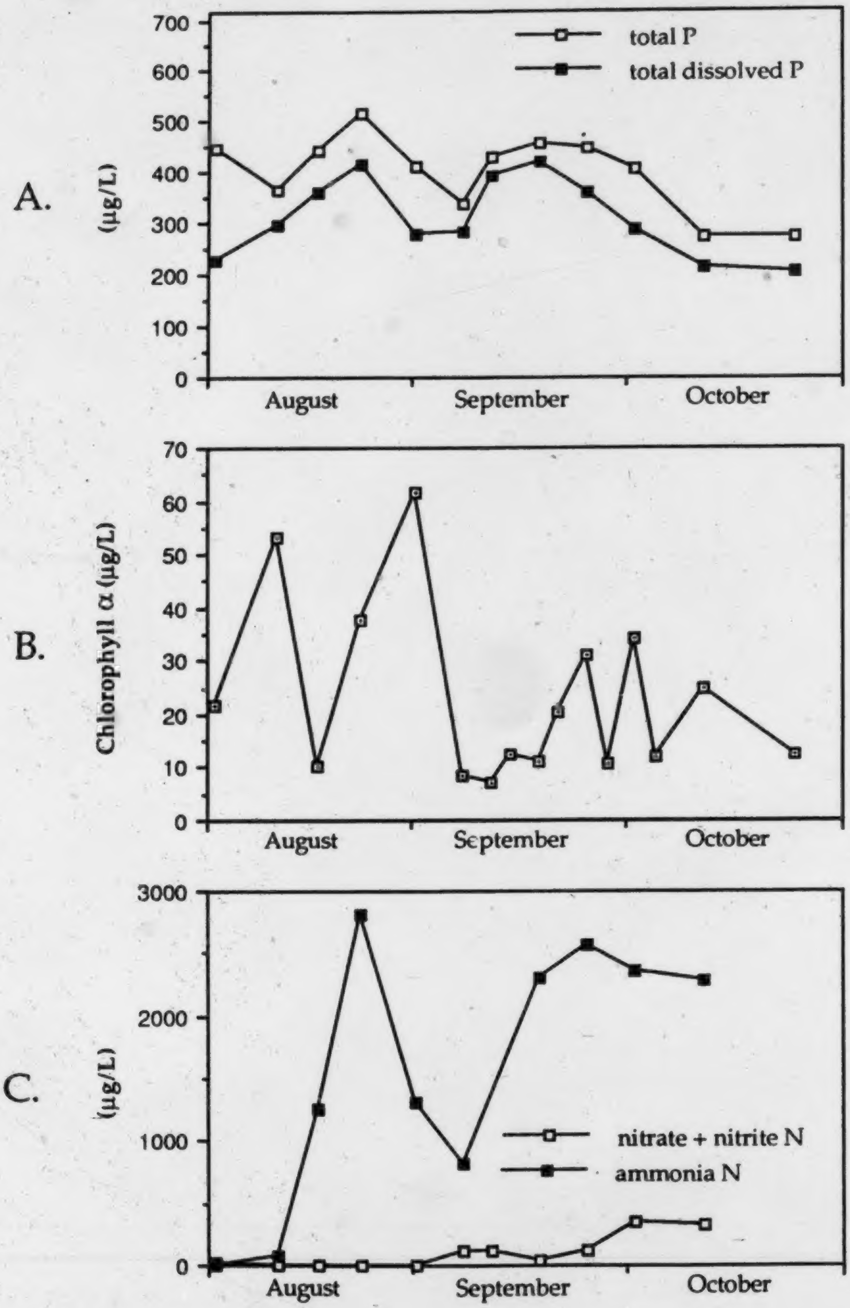


Figure 5.20 Little Beaver Lake
A. Total Phosphorus, Total Dissolved Phosphorus
B. Chlorophyll α Concentrations
C. Nitrate + Nitrite and Ammonia N

nitrogen levels decrease. All chlorophyll, phosphorous and nitrogen data are tabulated in the appendix

5.2.3.2 Water Treatment Plant Samples

The concern surrounding the human health effects due to long-term ingestion of water containing low levels of algal hepatotoxins warranted the study of water samples taken from the lakes which serve as raw water sources. These include Coal Lake which supplies the City of Wetaskiwin water treatment plant, Driedmeat Lake which supplies the City of Camrose water treatment plant and Little Beaver Lake which supplies the Village of Ferintosh water treatment plant. Water samples were taken at the raw water intake line and the final treated water line. These samples were immediately filtered and analysed for the hepatotoxin LR concentration in the water as well as the dissolved organic carbon concentrations. There was only one raw water sample (Coal Lake, Aug. 23) which contained LR concentrations above the detection limit of 1 ppb, and none of the treated water samples indicated that LR had remained in the water after treatment. These monitoring results are summarized in Table 5.4.

The results indicated that the raw water reaching the intake for the treatment plants did not contain detectable toxin levels. The dissolved organic carbon levels are also summarized in Table 5.4 but they cannot be related to the non-detectable toxin levels. Figures 5.21, 5.22 and 5.23 illustrate the water treatment processes in use at each of the three plants.

Table 5.4 Water Treatment Plant Samples

	Date Collected	DOC (ppm)	Microcystin LR (µg/L)
Camrose Raw	Aug-23-90	16.5	N/D
	Aug-27-90	16.5	N/D
	Sept-04-90	16.4	N/D
	Sept-10-90	15.4	N/D
	Sept-17-90	15.2	N/D
	Sept-24-90	13.9	N/D
	Oct-01-90	13.4	N/D
	Oct-09-90	17.1	N/D
Camrose Treated	Aug-23-90	7.2	N/D
	Aug-27-90	7.5	N/D
	Sept-04-90	7.2	N/D
	Sept-10-90	7.1	N/D
	Sept-17-90	7.0	N/D
	Sept-24-90	6.8	N/D
	Oct-01-90	6.3	N/D
	Oct-09-90	7.4	N/D
Wetaskiwin Raw	Aug-23-90	17.3	1
	Aug-27-90	17.3	N/D
	Sept-04-90	18.2	N/D
	Sept-10-90	24.1	N/D
	Sept-17-90	17.3	N/D
	Sept-24-90	24.9	N/D
	Oct-01-90	17.0	N/D
	Oct-09-90	17.9	N/D
Wetaskiwin Treated	Aug-23-90	7.9	N/D
	Aug-27-90	6.9	N/D
	Sept-04-90	6.4	N/D
	Sept-10-90	7.4	N/D
	Sept-17-90	7.1	N/D
	Sept-24-90	8.2	N/D
	Oct-01-90	9.8	N/D
	Oct-09-90	9.7	N/D
Ferintosh Raw	Aug-23-90	30.0	N/D
	Aug-27-90	30.8	N/D
	Sept-04-90	30.7	N/D
	Sept-10-90	31.6	N/D
	Sept-17-90	28.7	N/D
	Sept-24-90	27.4	N/D
	Oct-01-90	27.4	N/D
	Oct-09-90	28.1	N/D
Ferintosh Treated	Aug-23-90	23.5	N/D
	Aug-27-90	22.9	N/D
	Sept-04-90	20.9	N/D
	Sept-10-90	21.2	N/D
	Sept-17-90	20.8	N/D
	Sept-24-90	21.1	N/D
	Oct-01-90	20.3	N/D
	Oct-09-90	20.5	N/D

N/D = Not Detectable

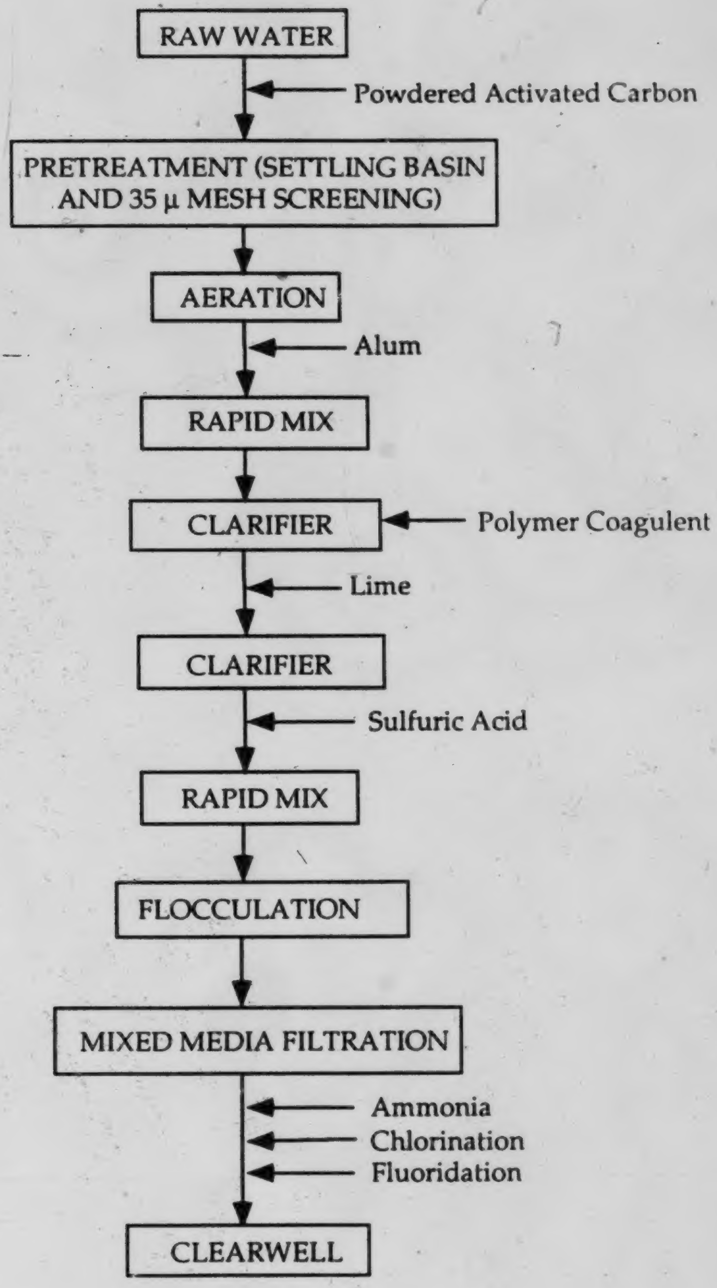

RAW WATER
Powdered Activated Carbon
PRETREATMENT (SETTLING BASIN AND 35 μ MESH SCREENING)
AERATION
Alum
RAPID MIX
CLARIFIER
Polymer Coagulent
Lime
CLARIFIER
Sulfuric Acid
RAPID MIX
FLOCCULATION
MIXED MEDIA FILTRATION
Ammonia
Chlorination
Fluoridation
CLEARWELL

Figure 5.22 Camrose Water Treatment Plant Process Flowsheet

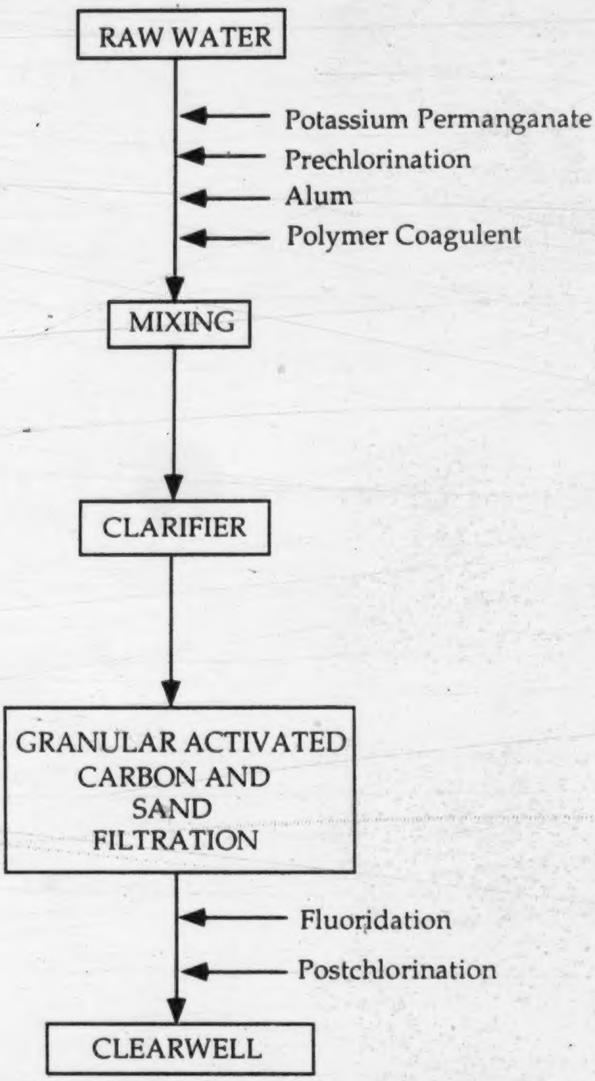

RAW WATER
Potassium Permanganate
Prechlorination
Alum
Polymer Coagulent
MIXING
CLARIFIER
GRANULAR ACTIVATED CARBON AND SAND FILTRATION
Fluoridation
Postchlorination
CLEARWELL

Figure 5.21 Wetaskiwin Water Treatment Plant Process Flowsheet

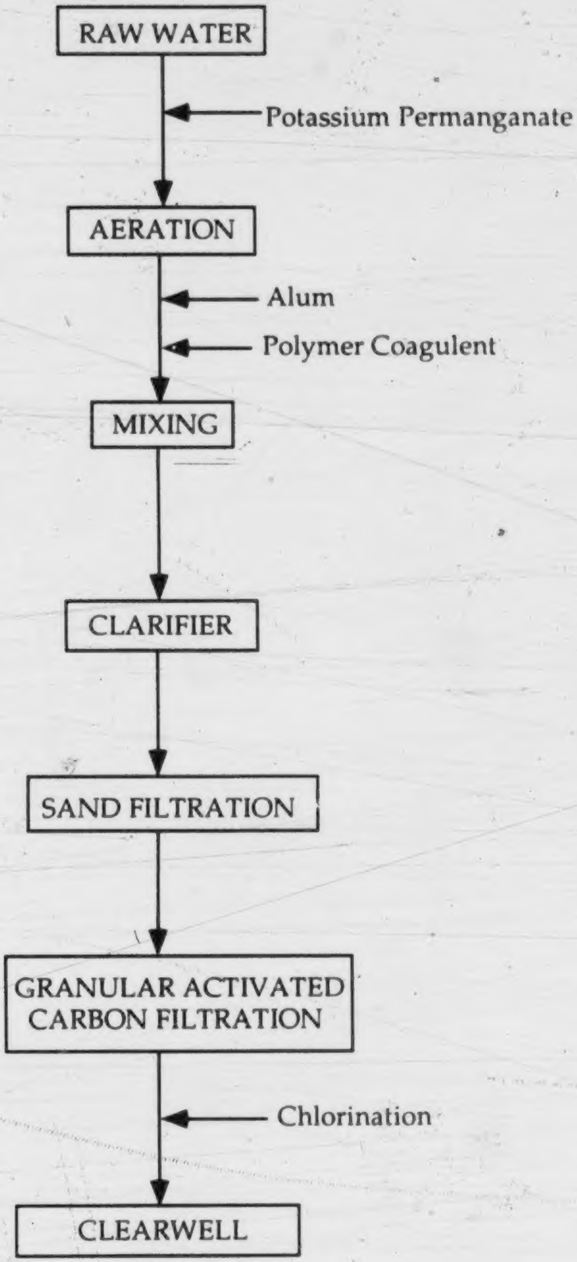

RAW WATER
Potassium Permanganate
AERATION
Alum
Polymer Coagulent
MIXING
CLARIFIER
SAND FILTRATION
GRANULAR ACTIVATED CARBON FILTRATION
Chlorination
CLEARWELL

Figure 5.23 Ferintosh Water Treatment Plant Process Flowsheet

The Wetaskiwin and Ferintosh treatment plants use GAC filtration and therefore, under correct operating procedures both would be expected to remove cyanobacterial toxins. The Camrose treatment plant utilizes PAC and would not likely be as reliable, given that practical PAC doses are usually less than 25 mg/L.

5.2.3.3 Bioassay Confirmation

The inherent weakness in the analytical methods used for the analysis of the algal toxins was that the compounds measured may not actually be toxic and they could be incorrectly labelled. To eliminate any such errors, and to demonstrate the urgency of the problems caused by these naturally produced toxins, a number of samples were chosen for toxicity confirmation through mouse bioassays.

Samples which were tested with the mouse bioassay were selected to include a range of measured toxin levels (toxin/g of biomass). Samples which contained varying levels of the hepatotoxin microcystin LR were found to cause acute liver damage which was strongly correlated to the measured toxin levels (Kotak 1990). The type of liver damage observed was consistent with that expected for microcystin LR and that demonstrated by the standard. None of the samples tested were found to cause any detectable neurological damage, thereby suggesting the absence of any substantial quantities of neurotoxins.

5.2.3.4 Mass Spectral Confirmation

Fast atom bombardment (FAB) mass spectral analysis was performed by Dr. Angelina Morales using a Kratos MS9 double focussing mass spectrometer. Samples were dissolved in approximately 1-µL of magic bullet matrix on the surface of the FAB probe tip. The probe was introduced into the ion source and exposed to the 7 KeV xenon atom beam. A clear pseudomolecular ion $(M+H)^+$ at m/z 995 was seen in the spectrum of each sample. No significant fragmentation can be expected with cyclic polypeptides, thus the spectra will show no significant fragment ions other than those attributable to the matrix. Spectra obtained for the isolated toxin and the microcystin LR standard are shown in the appendix. The isolated toxin was not desalted and thus some sodium salts are probably present. Additional pseudomolecular ions in the isolated toxin spectra, formed by clustering with sodium ($(M+Na)^+$ at m/z 1017, $(M+Na)^+Na$ at m/z 1040 and $(M+Na)^+Na_2$ at m/z 1063), were observed and reinforced confirmation of the microcystin LR molecular weight of 994 g/mole.

The intensities and isotope patterns in the pseudomolecular ion regions of both spectra are practically identical. The pseudomolecular region of the spectra of the isolated toxin is shown in Figure 5.24 and it compares favourably to this region of spectra obtained with the microcystin LR standard (Figure 5.25) and documented spectra of microcystin LR (Harada et al. 1988 and Krishnamurthy et al. 1986), again confirmation the isolation of microcystin LR from the algal bloom material.

5.2.4 Anatoxin-a

Samples which contained *Anabaena flos-aquae* cells were extracted and analysed for anatoxin-a. None of the natural algal cell samples collected contained detectable levels of anatoxin-a, but one sample which was cultured in the Department of Zoology from *Anabaena flos-aquae* cells which had been collected from the Frey dugout (Peace River area) did contain the neurotoxin. The area ratio for the standard m/z = 165 ion peak relative to the

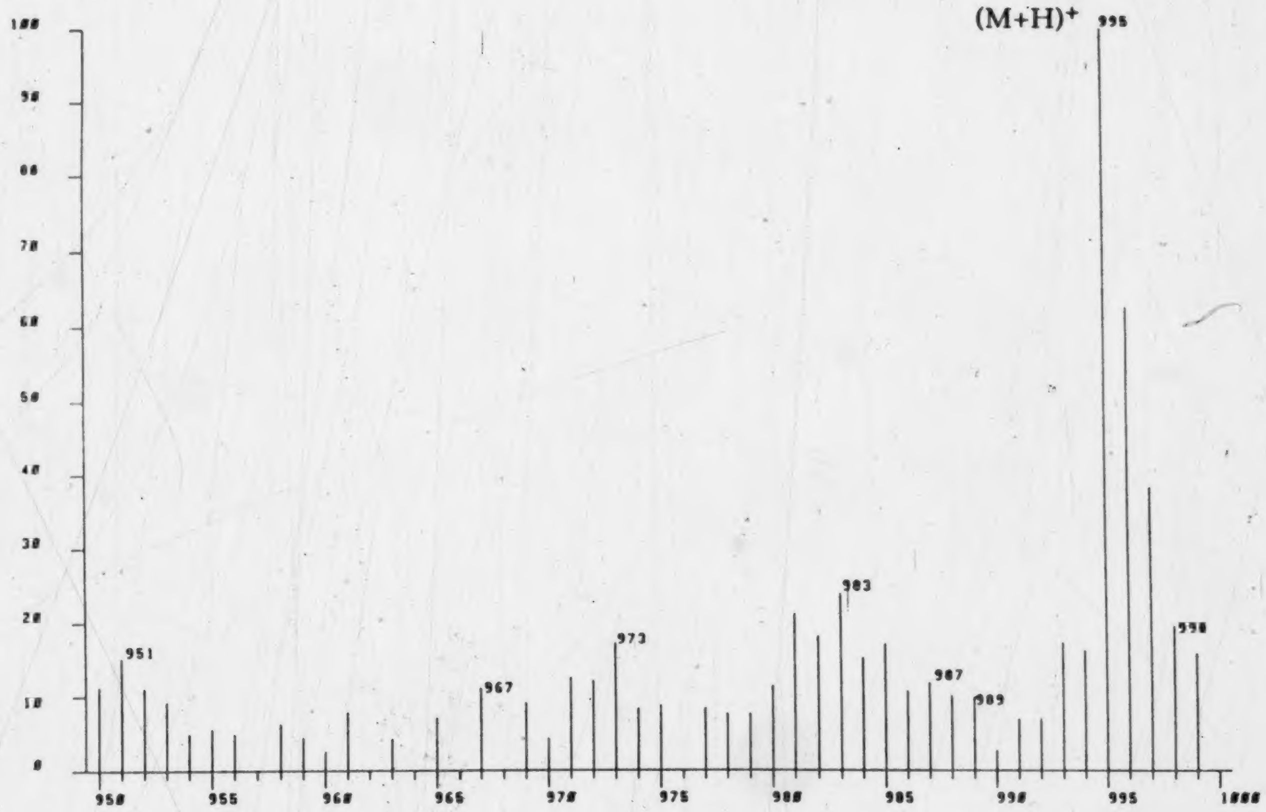


Figure 5.24 Pseudomolecular Ion Region of FAB Spectra of Isolated Microcystin LR in Magic Bullet Matrix

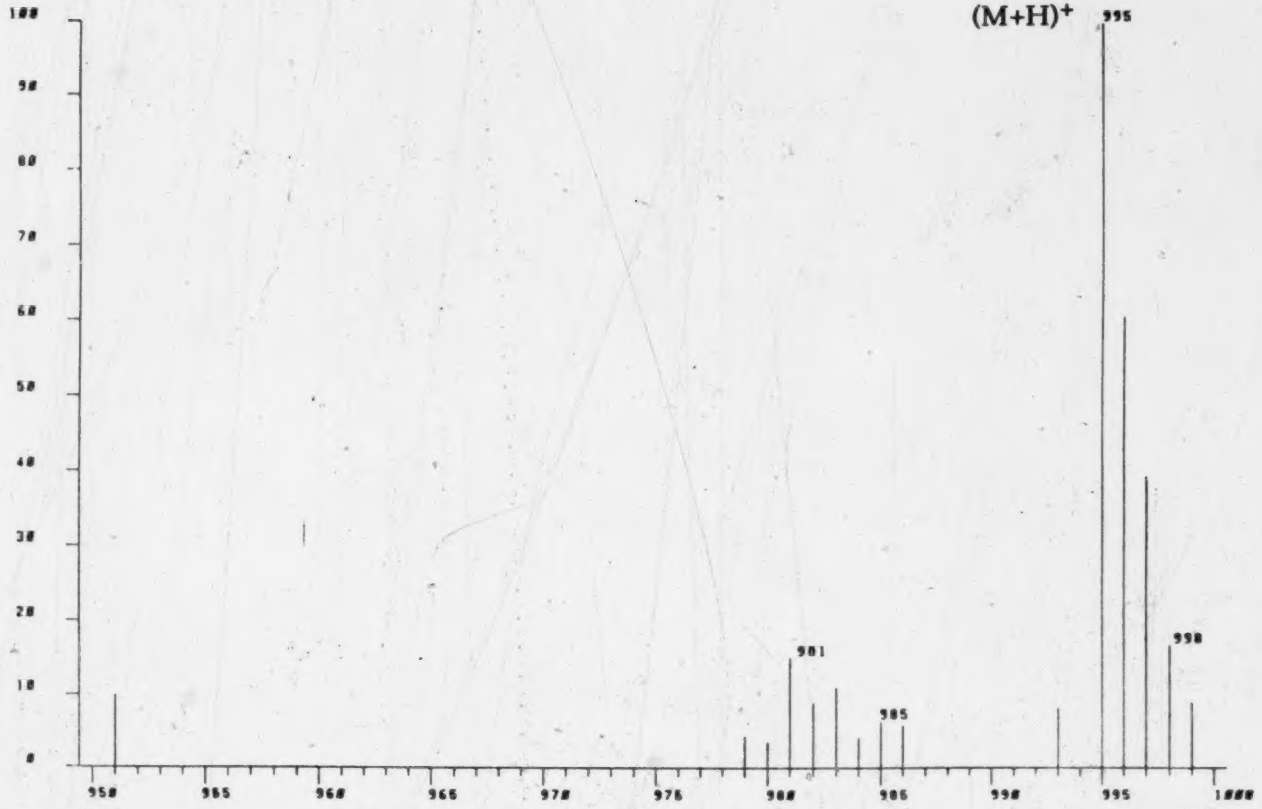


Figure 5.25 Pseudomolecular Ion Region of FAB Spectra of Microcystin LR Standard in Magic Bullet Matrix

internal standard m/z = 84 ion peak was .0025. This ratio corresponds to a GC injection of 1.0 ng of anatoxin-a, indicating that 1.0 µg was the total extracted mass of anatoxin-a. The extraction was carried out on 15.9 mg of biomass, so that there was 63 µg anatoxin-a per g of biomass. This analysis also indicated that the detection limit of the analytical method used was at least 1 ng of injected anatoxin-a.

5.2.5 Odour Analysis and Correlation

There is an understanding that odour incidents in water supplies are often related to the incidence of dense algal blooms. Along with this reality goes the possible misconception that, in the absence of any objectionable tastes or odours, there is also an absence of the toxic compounds produced by the algae. The samples collected from the study lakes were analysed for microcystin LR, anatoxin-a and the odour-causing agents geosmin, 2-MIB, and β-cyclocitral.

The only toxin found in our survey was microcystin LR, but the source, *Microcystis aeruginosa*, has not been reported to produce either geosmin or 2-MIB. Consequently, a correlation between microcystin LR occurrence and these two potent odour agents would not be predictable from the literature. However, *Microcystis aeruginosa* was reported to be a potent producer of β-cyclocitral (Jüttner 1988), so a correlation of microcystin LR with this compound is possible.

The data collected over the summer of 1990 in 8 Alberta lakes are summarized in Table 5.5 to 5.8. This data is based entirely on the presence or absence of FID peaks at retention times equivalent to standards of the target odour compounds. The protocol used for geosmin and 2-MIB was not sufficiently sensitive to detect these compounds, instrumentally, below their

Table 5.5 Coal Lake Toxin and Possible Target Odour Compounds (Peaks Detected by GC/FID at Correct Retention Time)

Date	Mass LR/Biomass (μg/g)	Geosmin*	2-MIB*	β-cyclocitral*
1-Aug-90	189	-	-	-
10-Aug-90	507	-	-	-
16-Aug-90	490	-	-	-
22-Aug-90	387	-	-	-
30-Aug-90	418	+	+	-
10-Sep-90	170	+	-	-
13-Sep-90	124	+	+	-
17-Sep-90	219	-	+	-
20-Sep-90	49	+	-	-
24-Sep-90	98	+	+	-
27-Sep-90	65	+	+	-
1-Oct-90	179	+	+	-
4-Oct-90	53	+	+	-
11-Oct-90	6	+	+	-

* (+) peak present; (-) peak absent

Table 5.6 Driedmeat Lake Toxin and Possible Target Odour Compounds (Peaks Detected by GC/FID at Correct Retention Time)

Date	Mass LR/Biomass (μg/g)	Geosmin*	2-MIB*	β-cyclocitral*
1-Aug-90	87	-	-	-
10-Aug-90	17	-	-	-
16-Aug-90	177	-	+	-
10-Sep-90	372	+	+	-
13-Sep-90	245	+	+	-
17-Sep-90	199	-	+	-
20-Sep-90	N/D	+	+	-
24-Sep-90	N/D	+	+	-
27-Sep-90	N/D	-	+	-

* (+) peak present; (-) peak absent

Table 5.7 Little Beaver Lake Toxin and Possible Target Odour Compounds
(Peaks Detected by GC/FID at Correct Retention Time)

Date	Mass LR/Biomass (μg/g)	Geosmin*	2-MIB*	β–cyclocitral*
1-Aug-90	8	-	+	-
10-Aug-90	24	+	+	-
16-Aug-90	12	+	+	-
22-Aug-90	34	-	+	-
30-Aug-90	53	+	-	-
6-Aug-90	N/D	+	+	-
10-Sep-90	20	+	+	-
13-Sep-90	27	+	+	-
17-Sep-90	N/D	+	+	-
20-Sep-90	209	+	+	-
24-Sep-90	435	+	+	-
27-Sep-90	74	+	+	-
1-Oct-90	30	-	-	-
4-Oct-90	25	+	+	-
11-Oct-90	4	-	-	-

* (+) peak present; (-) peak absent

Table 5.8 Other Lake Toxin and Possible Target Odour Compounds
(Peaks Detected by GC/FID at Correct Retention Time)

Lake (Date)	Mass LR/Biomass (μg/g)	Geosmin*	2-MIB*	β–cyclocitral*
Hastings (31-Jul-90)	8	+	+	-
Cooking (31-Jul-90)	N/D	-	-	-
Nakamun (2-Aug-90	39	-	-	-
La Nonne (2-Aug-90)	N/D	-	-	-
Isle (19-Aug-90)	244	+	+	-

* (+) peak present; (-) peak absent

odour threshold concentrations in water. The FID method for β-cyclocitral had a detection limit of 0.5 μg/L, well below the odour threshold concentration of 19 μg/L (Persson and Jüttner 1983). A limited set of samples were subjected to chromatographic sniffing which allowed consistent detection of geosmin and 2-MIB to equivalent water concentrations of 7 and 4 ng/L respectively. Chromatographic sniffing is more definitive than FID analysis based solely on retention time, but sniffing is not quantitative. Table 5.9 summarizes the results of the presence of the target odour compounds detected by chromatographic sniffing of water sample extracts in relation to microcystin LR analysis of bloom samples.

Table 5.9 Presence of Odour Agents (Detected by Chromatographic Sniffing) in Relation to Occurrence of Microcystin LR

Microcystin LR (μg/g biomass)	Geosmin*	2-Methyl-isoborneol*	Sample Source
N.D.	+	+	Little Beaver Lake
N.D.	+	+	Little Beaver Lake
20	+	+	Little Beaver Lake
27	+	+	Little Beaver Lake
53	+	+	Little Beaver Lake
87	-	-	Driedmeat Lake
170	-	+	Coal Lake
177	-	+	Driedmeat Lake
209	+	+	Little Beaver Lake
435	+	+	Little Beaver Lake
507	-	-	Coal Lake

* (+) compound detected; (-) compound not detected

The presence of an FID peak often failed to correspond to an odour peak during chromatographic sniffing suggesting interference by non-target compounds on the FID chromatograms. The water samples screened for target odour compounds by chromatographic sniffing indicated that geosmin and 2-MIB were consistently detectable at Little Beaver Lake while microcystin LR appeared in the bloom biomass to varying degrees. For water samples screened from Coal and Driedmeat Lakes by chromatographic sniffing, geosmin was not detectable and 2-MIB was detected in half of the samples while substantial quantities of microcystin LR were present in the bloom biomass. β-cyclocitral, which should be characteristic of *M. aeruginosa* was not detected in any of the water samples although 36 out of 43 bloom biomass samples from all sources were positive for microcystin LR. The results indicate that the odour agents monitored for water samples were not a reliable indicator of blue-green algal toxin in the biomass.

5.2.6 Evaluation of Significance

The toxin levels which may be present in the raw water supplies will vary. Possible loading scenarios for the lakes can be approximated using the chlorophyll α data and the microcystin LR toxin levels. The worst possible scenario during the summer of 1990 was at Coal Lake on August 20. With the summer average chlorophyll α value (to account for the spatial heterogeneity of the algal cell distribution) of 30 μg/L (wet weight) and average conversion factors, the average chlorophyll α concentration (dry weight) can be estimated:

$$C = \frac{\alpha M}{m} \qquad (2)$$

where: C = average biomass concentration (dry weight) (μg/L)
α = chlorophyll α concentration (wet weight) (μg /L)
M = mass of average dry cyanobacterial cell (pg)
m = mass of chlorophyll α per average cyanobacterial cell (pg)

$$C = \frac{30\ \mu g/L * 32\ pg}{0.36\ pg}$$

$$C = 2 \times 10^3\ \mu g/L$$

or: $$C = 0.002\ g/L$$

This biomass concentration (dry weight) can then be used with the microcystin LR concentration (dry weight) of 507 μg/g to estimate the toxin loading:

$$LR = CM \qquad (3)$$

where: LR = microcystin LR loading (μg/L)
C = average biomass concentration (dry wight) (g/L)
M = microcystin LR concentration (μg toxin / dry g biomass)

$$LR = 0.002\ g/L * 507\ \mu g/g$$

$$LR = 1\ \mu g/L$$

In this case there would be an approximate microcystin LR loading of 1 μg toxin/L of lake water. If the bloom collapsed rapidly, concentrations at this level could be found in the water. Concentrations reaching the water treatment plant submerged intake would be this high if there was complete vertical mixing. This could be possible because the toxins will be released as the cells settle and therefore can be distributed at all depths. Vertical stratification could be expected in deep lakes.

The highest toxin loading of Driedmeat Lake was on September 10 when the toxin concentration could have reached 0.4 μg microcystin LR/L of lake water, if there had been a rapid collapse. In Little Beaver Lake the highest concentration would have been on September 24 when a rapid collapse would have resulted in 1 μg microcystin LR/L of lake water.

The above values were calculated assuming a homogeneous mix of algal cells throughout the lake water. Cyanobacteria have gas vacuoles which allow them to float on or near the surface, and under certain wind conditions algae will collect on the windward side of the lake in a dense scum on top of the water. If such a scum collected directly over the water intake at the time of a rapid collapse, there would be considerably higher toxin levels entering the water treatment plant intake.

The thickness of the algal cells used in the batch studies can be taken as a pessimistic upper bound for this type of scum. The batch study vessels had cross sectional areas of 0.045 m^2 and each contained approximately 15 g of algal cells (dry weight). However, the batch study vessel unnaturally confined the bloom material. Figure 4.10 indicates that a natural bloom has approximately 50% coverage of biomass which is about half as thick as biomass confined to a batch study vessel. Thus a natural bloom over the 0.45 m^2 area can be estimated as having only one quarter the biomass (i.e. 3.75 g dry weight). If this algal suspension collected directly over the withdrawal area of an intake which was at a depth of 3 m, the biomass concentration can be estimated as:

$$C = \frac{m}{A\,d} \tag{4}$$

where:
C = biomass concentration (g/m^3)
m = mass of cells
A = area of vessel (m^2)
d = depth of theoretical intake (m)

$$C = \frac{3.75\ \mathrm{g}}{0.045\ \mathrm{m}^2 * 3\ \mathrm{m}}$$

$$C = 27.8\ \mathrm{g/m^3}$$

or $$C = 0.0278\ \mathrm{g/L}$$

If these cells contained microcystin LR at a concentration of 400 µg/g, equation (2) can be used to estimate the microcystin LR loading:

$$LR = 0.0278\mathrm{g/L} * 400\ \mu\mathrm{g/g}$$

$$LR = 11\ \mu\mathrm{g/L}$$

If there was a rapid collapse of the bloom, there could be a complete mix water column concentration of 11 µg LR/L. This was once again assuming complete vertical mixing because the toxin is released as the dead cells are settling.

If this was the raw water for a treatment plant utilizing PAC, 90% of the toxin would be removed leaving 1.1 µg/L of toxin remaining in the treated water. The hazard associated with the level is difficult to judge because specific human health guidelines for microcystin LR have not been developed. However an approximation can be inferred from the approach taken to develop standards for acute poisons. Given the LD_{50} of 50 µg/kg reported for microcystin LR (Carmichael 1988), a no effect level would likely be at least 10 fold lower. If an uncertainty factor of 10 for species extrapolation (mice to humans) and another uncertainty factor of 10 for most sensitive individual, a tolerable acute dose for humans might be approximated at 0.050 µg/kg. A common exposure assessment for a 20 kg child would assume consumption of 1.4 L/d. In the case of microcystin LR the estimated "safe" level for acute poisoning corresponds to a water concentration of 0.7 µg/L.

Consequently, the worst case calculation above suggests that an estimated safe level could be substantially exceeded. The calculations also show that an estimated "safe" level would be just below our current detection limit for the toxin. These assumptions relate to the safety relative to acute lethal poisoning. A safe level for chronic liver damage may be substantially lower. Liver damage can occur in individuals whose drinking water supplies are contaminated with toxic cyanobacteria (Falconer et al. 1983a and Hawkins et al. 1985). The possible human health effects and the estimated worst case scenario calculations outlined above, indicate the importance of monitoring the cyanobacteria found in drinking water supplies.

6. CONCLUSIONS

The preliminary work performed for this project led to a number of distinct conclusions and each of the three research objectives was met during the course of this research.

Laboratory batch experiments with field samples of cyanobacterial bloom material showed that copper sulfate lysed cells, leading to catastrophic release of microcystin LR to the water phase. Lime treatment tended to precipitate cells but did not cause extensive cell lysis or toxin release. Analysis of the water following toxin release showed a half life for microcystin LR, under artificial laboratory conditions, of 2.8 days. Based on these observations, copper sulfate should not be used to treat potentially toxic cyanobacterial blooms in water to be consumed by humans or animals. These findings should be understood by operators of water treatment systems drawing upon water sources vulnerable to cyanobacterial blooms.

The survey of various water bodies throughout Alberta demonstrated that toxic cyanobacterial blooms were prevalent during the summer of 1990. The potent hepatotoxin, microcystin LR, was prevalent in cyanobacterial blooms on three shallow lakes used as municipal drinking water sources. Although no toxic blooms were detected in the agricultural dugouts sampled, this was likely due to active suppression of the algal blooms by chemical treatments.

Some common cyanobacterial odour agents (geosmin, 2-MIB, and β-cyclocitral) failed to provide either sensitive or reliable warning of the occurrence of microcystin LR in cyanobacterial blooms on the lakes studied. The absence of a relationship between odour agents, geosmin and 2-MIB, and the presence of microcystin LR is consistent with the absence of any literature reports of *Microcystis* spp. producing geosmin or 2-MIB. However, β-cyclocitral, which should be characteristic of *Microcystis aeruginosa,* also failed to be an indicator of microcystin LR presence.

Toxic blooms were found in a number of lakes which serve as raw water sources for municipal water treatment plants. Calculations, based upon the biomass concentrations of Microcystin LR measured in this survey, suggest that treated water, under plausible worst case bloom collapse conditions, might not be safe by an adequate margin for the most sensitive human consumers. Given the prevalence of eutrophic water supply sources and the absence of any apparent relationship between odour and toxin occurrence, cyanobacterial toxins warrant serious consideration quite separately from the valid concerns about odour.

7. RECOMMENDATIONS

Because this work was only the preliminary investigation of blue-green algal problems in prairie water supplies there are a number of factors which must be studied to gain a more complete understanding of the situation.

A complete study of the environmental problems associated with algal blooms should include identification and quantification of the critical chemical and environmental properties required to accurately predict the distribution and ultimate fate of the algal toxins, as well as the health risks associated with the toxins. The known properties of the *Microcystis* and *Anabaena* toxins are limited and a determination of these properties was not feasible during this study because only extremely limited quantities of the toxins were available for study.

The most critical physical property in the study of the environmental distribution of the toxic compounds is aqueous solubility. The solubility of the cyanobacterial toxins did not limit the studies carried out for this preliminary investigation, but it could influence more detailed studies of the environmental properties of the toxins. Properties which are indicative of the transport or partitioning of the toxins within and between environmental compartments also deserve consideration. Distribution is expected to be limited to interactions between water and biota or water and sediment because the toxins are not volatile and can be adsorbed on activated carbon. The tendency of the toxins to accumulate in each phase could be inferred from knowledge of the octanol/water partition coefficient.

Understanding the persistence of the compounds in the aquatic environment is also critical. Degradation of the polypeptide toxins may occur through hydrolysis, photolysis or biodegradation (aerobic or anaerobic). Biodegradation studies could be performed if photolysis and hydrolysis were not found to be significant degradation pathways, but there would be some difficulty in setting up biodegradation conditions in the laboratory which simulate environmental conditions. In all degradation studies it will be critical to consider the effect of key environmental parameters such as temperature, concentration, pH and ionic strength.

There should also be more detailed study into the release of toxins after treatment with lime, copper sulfate and other control chemicals in the natural environment, in order to include the effects of mixing and establish decomposition mechanisms. Studies using ^{14}C-labeled cyanobacterial cultures (Fedorak and Huck 1988) could be very informative.

This survey and the corresponding theoretical toxin loading calculations suggest there is a need for a drinking water guideline to be developed for microcystin LR.

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APPENDIX

DECONTAMINATION AND DISPOSAL

Decontamination Procedure

Inactivation of cyanobacterial toxins is achieved through exposure to strong oxidizing agents such as 1.5% NaOCl (0.002 M) for 30 min. Spills of solutions of cyanobacterial toxins should be adsorbed with spill pillows or paper towels, and the contaminated materials should be soaked in 1.5% NaOCl for at least 30 min. The site of the spill should be thoroughly washed with NaOCl and the area should then be washed with soap or detergent and plenty of water. All contaminated glassware must be soaked in NaOCl prior to washing and all working surfaces must be washed with NaOCl daily.

Disposal Procedures

The waste from these experiments is anticipated to be small. Solutions of the cyanobacterial toxins must be exposed to 1.5% NaOCl for at least 30 min prior to disposal.

ANALYTICAL DATA

Table A1 Control Batch Studies

Study #1 Control 7.9 g total biomass

Time (Hour)	Total Volume (L)	pH	D.O. (mg/L)	D.O.C. (ppm)	Chlor-a	T.D.P. (µg/L)	Microcystin LR (mg in aqueous)
0	10	8.15	4.50	16.6	31.88	60	0.043
1	9.3	8.19	4.35	16.7	96.08	1468	0.041
2	8.6	8.23	4.35	16.1	88.81	334	0.038
4	7.9	8.23	4.00	17.2	42.28	76	0.035
8	7.2	8.20	3.00	16.7	18.91	61	0.032
24	6.5	7.86	1.80	19.6	68.26	240	0.03
48	5.8	7.39	1.00	32.2	84.45	728	0.091
72	5.1	7.26	0.90	37.8	92.45	734	0.145
240	4.7	7.95	0.30	49.9	825.67	473	0.044

Study #2 Control 14.78 g total biomass

Time (days)	Total Volume (L)	pH	D.O. (mg/L)	D.O.C. (ppm)	Chlor-a (µg/L)	T.D.P. (µg/L)	Microcystin LR (mg in aqueous)
0	10.7	7.63	3.10	39.2	407.61	166	0.051
1.167	10	6.98	1.80	71.2	426.27	1186	0.369
2.083	9.3	6.90	1.05	91.2	388.50	1695	0.553
3	8.6	6.81	0.90	97.9	380.87	1464	0.332
4	7.9	6.70	0.60	98.9	362.05	1500	0.226
5	7.2	6.65	0.50	106.1	262.18	1726	0.187
7	6.5	6.70	0.35	103.3	337.67	1813	0.148
10	5.8	6.71	0.30	75.9	305.59	1984	0.119

Study #3 Control 18.79 g total biomass

Time (days)	Total Volume (L)	pH	D.O. (mg/L)	D.O.C. (ppm)	Chlor-a (µg/L)	T.D.P. (µg/L)	Microcystin LR (mg in aqueous)
0	10.7	7.30	2.25	37.7	291.97	379	0.055
1	10	6.72	1.90	73.4	327.01	1195	0.39
2	9.3	6.39	1.25	103.9	392.19	1713	0.625
3.042	8.6	6.40	1.00	120.1	484.22	1954	N/A
4.042	7.9	6.47	0.90	120.4	485.73	2807	0.389
5	7.2	6.48	0.85	120.7	506.21	2102	0.354
6	6.5	6.55	0.75	117.1	514.85	2150	0.297
8	5.8	6.69	0.40	118.1	536.41	2183	0.214
10	5.1	6.75	0.30	105.2	582.16	2167	0.243

Table A2 Copper Sulfate Batch Studies

Study #1 2 mg/L CuSO4 added to 8.3 g total biomass

Time (Hour)	Total Volume (L)	pH	D.O. (mg/L)	T.O.C. (ppm)	Chlor-a (µg/L)	T.D.P. (µg/L)	Microcystin LR (mg in aqueous)
0	10	8.15	4.50	16.12	55.97	467	0.043
1	9.3	8.01	3.25	23.23	124.97	128	0.041
2	8.6	7.99	3.00	18.17	106.07	141	0.038
4	7.9	7.98	2.80	18.34	64.91	287	0.035
8	7.2	7.98	2.30	21.16	53.79	374	0.137
24	6.5	7.90	1.20	42.07	507.02	1013	1.475
48	5.8	7.75	0.90	54.24	542.05	1224	3.409
72	5.1	7.70	0.80	72.95	607.26	1333	3.599
240	4.7	7.9	0.3	80.68	930.15	2018	0.039

Study #2 2 mg/L CuSO4 added to 15.32 g total biomass

Time (days)	Total Volume (L)	pH	D.O. (mg/L)	D.O.C. (ppm)	Chlor-a (µg/L)	T.D.P. (µg/L)	Microcystin LR (mg in aqueous)
0	10.7	7.61	3.05	32.6	459.75	214	0.052
1.167	10	7.10	1.30	95.3	406.23	1161	1.466
2.083	9.3	6.90	1.10	N/A	487.57	2152	2.006
3	8.6	6.73	1.00	119.3	777.31	1353	1.504
4	7.9	6.51	0.95	96.4	743.99	1568	1.721
5	7.2	6.46	0.90	109.2	806.75	1544	1.036
7	6.5	6.50	0.80	110.2	733.92	1566	0.405
10	5.8	6.60	0.65	95.8	742.08	1932	0.359

Study #3 2 mg/L CuSO4 added to15.76 g total biomass

Time (days)	Total Volume (L)	pH	D.O. (mg/L)	D.O.C. (ppm)	Chlor-a (µg/L)	T.D.P. (µg/L)	Microcystin LR (mg in aqueous)
0	10.7	7.25	2.00	38.6	332.28	379	0.069
1	10	6.79	1.80	81.8	453.46	1069	0.791
2	9.3	6.42	1.15	104.7	591.20	1342	1.452
3.042	8.6	6.43	1.10	114.6	608.25	1456	N/A
4.042	7.9	6.48	1.15	112.5	650.58	2089	1.477
5	7.2	6.55	0.95	122.0	673.42	1696	1.226
6	6.5	6.61	0.90	109.2	678.30	1510	1.243
8	5.8	6.72	0.50	95.8	692.42	1619	0.519
10	5.1	6.73	0.40	108.3	693.09	2178	0.227

Table A3 Lime Batch Studies

Study #1 100 mg/L Ca(OH)2 added to 8.1g total biomass

Time (Hour)	Total Volume (L)	pH	D.O. (mg/L)	T.O.C. (ppm)	Chlor-a (µg/L)	T.D.P. (µg/L)	Microcystin LR (µg/L in aq.)
0	10	8.17	4.50	15.1	20.63	59	0
1	9.3	9.88	3.30	18.7	468.64	124	0
2	8.6	9.85	3.20	19.2	391.43	182	0
4	7.9	9.81	2.85	19.0	245.19	197	0
8	7.2	9.67	2.30	22.3	156.34	207	0.06
24	6.5	9.16	1.70	37.9	613.68	749	0.233
48	5.8	8.46	1.00	40.8	582.51	724	0.178
72	5.1	8.08	0.90	49.0	425.60	748	0.062
10	4.7	8.02	0.30	50.3	559.15	263	0.067

Study #2 100mg/L Ca(OH)2 added to15.98g total biomass

Time (days)	Total Volume (L)	pH	D.O. (mg/L)	D.O.C. (ppm)	Chlor-a (µg/L)	T.D.P. (µg/L)	Microcystin LR (mg in aqueous)
0	10.7	7.60	3.00	37.8	408.10	1596	0.059
1.167	10	8.47	1.60	69.7	451.02	842	0.202
2.083	9.3	7.98	1.10	81.4	482.96	1396	0.235
3	8.6	7.64	1.00	99.0	558.03	1214	0.212
4	7.9	7.40	0.95	91.5	531.70	1462	0.151
5	7.2	7.18	0.90	118.4	352.44	1375	0.145
7	6.5	7.08	0.80	118.7	516.58	1514	0.117
10	5.8	7.05	0.70	118.2	331.18	1396	0.088

Study #3 100 mg/L Ca(OH)2 added to16.25g total biomass

Time (days)	Total Volume (L)	pH	D.O. (mg/L)	D.O.C. (ppm)	Chlor-a (µg/L)	T.D.P. (µg/L)	Microcystin LR (mg in aqueous)
0	10.7	7.25	2.05	43.5	306.19	379	0.085
1	10	8.88	1.70	76.1	369.14	972	0.111
2	9.3	8.18	1.00	87.5	450.73	1044	0.146
3.042	8.6	7.97	0.95	87.2	483.71	1066	N/A
4.042	7.9	7.48	0.90	115.0	568.49	2011	0.155
5	7.2	7.40	0.80	118.1	543.00	1426	0.152
6	6.5	7.31	0.60	135.0	593.62	1524	0.163
8	5.8	7.20	0.35	141.0	738.48	1504	0.156
10	5.1	7.11	0.30	154.5	679.92	2241	0.098

Table A4 Central Alberta Weather, Edmonton International Airport.
(adapted from: Environment Canada 1990)

Date	Temperature °C high	Precipitation mm	Average Windspeed km/h	Bright Sun hours
July 1 1990	20.7	tr	10.2	6.9
July 2 1990	17.3	8.6	13.6	0.0
July 3 1990	13.5	75.6	27.6	0.0
July 4 1990	17.1		11.1	11.3
July 5 1990	21.5		20.7	13.9
July 6 1990	16.0	32.6	15.4	0.0
July 7 1990	21.8		7.7	12.8
July 8 1990	24.7		5.4	14.8
July 9 1990	24.6	0.4	12.3	12.8
July 10 1990	25.9		4.7	13.2
July 11 1990	27.4		14.5	15.4
July 12 1990	30.7	tr	13.0	14.2
July 13 1990	25.1		8.4	14.5
July 14 1990	22.2		16.4	14.7
July 15 1990	23.1		3.9	9.0
July 16 1990	18.8	22.6	9.3	5.3
July 17 1990	17.9	0.3	13.8	11.9
July 18 1990	17.8		8.2	12.4
July 19 1990	16.6	4.6	7.3	5.9
July 20 1990	18.7	tr	4.0	12.6
July 21 1990	22.2		4.8	15.1
July 22 1990	25.4	0.4	10.3	14.2
July 23 1990	26.6		6.8	12.9
July 24 1990	22.0		6.0	6.0
July 25 1990	25.2	tr	7.9	7.1
July 26 1990	20.2	2.8	2.2	0.3
July 27 1990	24.7		14.4	10.7
July 28 1990	21.8	1.4	8.9	15.0
July 29 1990	24.8	tr	7.2	14.1
July 30 1990	28.4		10.3	13.0
July 31 1990	27.6		7.2	12.8

Continued . . .

Table A4 Continued.

Date	Temperature °C high	Precipitation mm	Average Windspeed km/h	Bright Sun hours
Aug 1 1990	24.3		6.8	14.1
Aug 2 1990	20.0	3.8	11.1	11.4
Aug 3 1990	23.8		6.3	14.0
Aug 4 1990	26.6		3.8	14.4
Aug 5 1990	30.7		6.3	14.5
Aug 6 1990	33.0		10.4	11.9
Aug 7 1990	25.5		5.8	12.9
Aug 8 1990	24.4	tr	4.3	13.4
Aug 9 1990	26.7	1.2	6.8	11.4
Aug 10 1990	27.6		7.3	13.5
Aug 11 1990	27.3		5.7	12.8
Aug 12 1990	29.4		6.6	12.8
Aug 13 1990	22.2		10.1	7.2
Aug 14 1990	25.2	0.2	13.1	5.2
Aug 15 1990	24.3	tr	11.2	9.0
Aug 16 1990	17.0	23.0	15.2	0.3
Aug 17 1990	17.3	0.2	13.1	0.3
Aug 18 1990	17.5	1.6	14.0	2.0
Aug 19 1990	24.4		6.4	10.8
Aug 20 1990	27.8		16.8	13.5
Aug 21 1990	28.0		8.5	7.0
Aug 22 1990	21.5	2.0	11.8	2.3
Aug 23 1990	14.7	0.4	15.9	1.1
Aug 24 1990	11.8		5.7	2.4
Aug 25 1990	14.2	0.4	7.1	0.2
Aug 26 1990	13.7	4.4	6.2	3.1
Aug 27 1990	21.1		12.2	12.2
Aug 28 1990	25.2		8.8	12.9
Aug 29 1990	25.0		10.3	13.0
Aug 30 1990	20.6	8.4	23.8	0.2
Aug 31 1990	11.9	18.6	11.9	0.0

Continued . . .

Table A4 Concluded.

Date	Temperature °C high	Precipitation mm	Average Windspeed km/h	Bright Sun hours
Sept 1 1990	20.5		9.1	10.9
Sept 2 1990	19.2		10.7	11.7
Sept 3 1990	18.3	1.4	9.7	11.8
Sept 4 1990	23.6		11.7	12.2
Sept 5 1990	22.4		8.8	11.5
Sept 6 1990	23.8		11.0	11.0
Sept 7 1990	25.4	tr	15.8	10.6
Sept 8 1990	15.0	1.2	11.0	3.0
Sept 9 1990	21.9		9.7	9.9
Sept 10 1990	25.4		11.1	11.3
Sept 11 1990	24.5		12.8	10.5
Sept 12 1990	16.6	0.6	19.4	3.0
Sept 13 1990	22.3		13.6	10.5
Sept 14 1990	22.4		12.4	10.7
Sept 15 1990	25.5		22.6	10.7
Sept 16 1990	21.3	0.8	23.6	5.6
Sept 17 1990	16.1	tr	19.8	10.7
Sept 18 1990	22.6		10.4	10.2
Sept 19 1990	17.1	0.2	17.9	7.9
Sept 20 1990	16.8	tr	8.5	9.2
Sept 21 1990	16.6	tr	15.7	10.3
Sept 22 1990	25.8		15.8	10.0
Sept 23 1990	28.6		13.5	10.1
Sept 24 1990	29.1		13.7	10.1
Sept 25 1990	22.5		12.3	8.6
Sept 26 1990	16.4		7.7	7.1
Sept 27 1990	13.7		7 9	7.9
Sept 28 1990	16.4		22.9	7.1
Sept 29 1990	14.3		14.5	6.6
Sept 30 1990	16.7		11.2	9.7
Oct 1 1990	15.1		13.9	0.4
Oct 2 1990	11.5	tr	27.8	7.3
Oct 3 1990	9.3	tr	18.9	7.4
Oct 4 1990	1.2	6.6 (snow)	11.0	0.0

Table A5 Camrose Area Weather (adapted from: Environment Canada, unpub. data 1990) and Driedmeat Lake Water Temperature

Date	Temperature °C high (Camrose)	Precipitation mm (Camrose)	Water Temp. °C (Dried Meat Lake)
July 1 1990	23.0		
July 2 1990	20.5	45.2	
July 3 1990	12.0	31.8	
July 4 1990	16.5		
July 5 1990	21.0	9.8	
July 6 1990	14.5	38.4	
July 7 1990	21.5		
July 8 1990	24.5		
July 9 1990	24.0	0.8	
July 10 1990	26.0		
July 11 1990	27.0		
July 12 1990	30.5		
July 13 1990	24.5		
July 14 1990	22.0		
July 15 1990	24.0	0.4	
July 16 1990	18.5	24.2	
July 17 1990	17.5		
July 18 1990	17.5		
July 19 1990	17.5	tr	
July 20 1990	17.5		
July 21 1990	21.5		
July 22 1990	24.5		
July 23 1990	27.0		
July 24 1990	23.0	0.6	
July 25 1990	25.5	2.0	
July 26 1990	19.0	4.2	
July 27 1990	24.5		
July 28 1990	22.5		
July 29 1990	23.0		
July 30 1990	27.5		
July 31 1990	27.5		

Continued . . .

Tabel A5 Continued.

Date	Temperature °C high (Camrose)	Precipitation mm (Camrose)	Water Temp. °C (Dried Meat Lake)
Aug 1 1990	25.0	6.8	22.0
Aug 2 1990	21.0		
Aug 3 1990	23.0		
Aug 4 1990	26.5		
Aug 5 1990	30.0		
Aug 6 1990	32.0		
Aug 7 1990	25.0	0.4	
Aug 8 1990	24.5		
Aug 9 1990	26.0		
Aug 10 1990	27.5		24.0
Aug 11 1990	27.0		
Aug 12 1990	24.5		
Aug 13 1990	23.5	0.6	
Aug 14 1990	23.5	1.4	
Aug 15 1990	24.5	4.4	
Aug 16 1990	15.0	5.4	21.5
Aug 17 1990	15.5	0.2	
Aug 18 1990	15.0	10.6	
Aug 19 1990	24.5		
Aug 20 1990	16.0		
Aug 21 1990	26.5		
Aug 22 1990	19.5	1.6	18.0
Aug 23 1990	12.5	1.2	
Aug 24 1990	12.5		
Aug 25 1990	15.5	0.8	
Aug 26 1990	13.0	10.0	
Aug 27 1990	20.0		
Aug 28 1990	24.0		
Aug 29 1990	25.0		
Aug 30 1990	16.5	10.4	
Aug 31 1990	13.0	6.0	

Continued . . .

Table A5 Concluded.

Date	Temperature °C high (Camrose)	Precipitation mm (Camrose)	Water Temp. °C (Dried Meat Lake)
Sept 1 1990	20.5		
Sept 2 1990	19.0		
Sept 3 1990	18.5		
Sept 4 1990	23.5		
Sept 5 1990	25.5		
Sept 6 1990	23.5		18.0
Sept 7 1990	26.0		
Sept 8 1990	13.0	4.2	
Sept 9 1990	22.5		
Sept 10 1990	24.5		17.0
Sept 11 1990	24.5		
Sept 12 1990	15.5	1.4	
Sept 13 1990	21.0		16.0
Sept 14 1990	23.0		
Sept 15 1990	25.0		
Sept 16 1990	24.0	tr	
Sept 17 1990	16.5		15.0
Sept 18 1990	22.5		
Sept 19 1990	17.5	tr	
Sept 20 1990	16.5		13.5
Sept 21 1990	16.5		
Sept 22 1990	24.0		
Sept 23 1990	28.5		
Sept 24 1990	28.0		14.5
Sept 25 1990	20.0		
Sept 26 1990	17.0		
Sept 27 1990	14.0	tr	13.5
Sept 28 1990	15.5		
Sept 29 1990	16.0		
Sept 30 1990	17.0		
Oct 1 1990	17.0		11.0
Oct 2 1990	11.5		
Oct 3 1990	9.0		
Oct 4 1990	2.0	15.8(snow)	

Table A6 Wetaskiwin Area Weather (adapted from: Environment Canada, unpub. data 1990) and Coal Lake Water Temperature.

Date	Temperature °C high (Wetaskiwin)	Precipitation mm (Wetaskiwin)	Water Temp. °C (Coal Lake)
July 1 1990	24.5	2.0	
July 2 1990	24.5	50.8	
July 3 1990	10.5	20.2	
July 4 1990	17.0		
July 5 1990	22.0	19.4	
July 6 1990	15.0	12.0	
July 7 1990	22.5		
July 8 1990	25.5		
July 9 1990	25.0	20.0	
July 10 1990	28.0		
July 11 1990	27.5		
July 12 1990	31.0	tr	
July 13 1990	26.0		
July 14 1990	26.0		
July 15 1990	24.5		
July 16 1990	19.5	21.2	
July 17 1990	18.0	tr	
July 18 1990	19.0		
July 19 1990	18.5	tr	
July 20 1990	19.0		
July 21 1990	23.0		
July 22 1990	25.0		
July 23 1990	28.0		
July 24 1990	23.0	0.4	
July 25 1990	25.5	8.4	
July 26 1990	21.0	7.8	
July 27 1990	25.0	tr	
July 28 1990	25.5		
July 29 1990	24.0		
July 30 1990	28.0		
July 31 1990	28.5		

Continued . . .

Table A6 Continued.

Date	Temperature °C high (Wetaskiwin)	Precipitation mm (Wetaskiwin)	Water Temp. °C (Coal Lake)
Aug 1 1990	25.0		22.0
Aug 2 1990	21.5	8.2	
Aug 3 1990	23.5	0.8	
Aug 4 1990	27.0		
Aug 5 1990	30.0		
Aug 6 1990	32.0		
Aug 7 1990	27.0		
Aug 8 1990	25.0		
Aug 9 1990	26.0		
Aug 10 1990	29.0		22.0
Aug 11 1990	28.5		
Aug 12 1990	29.0	1.0	
Aug 13 1990	21.5	3.0	
Aug 14 1990	25.0	tr	
Aug 15 1990	25.0	20.0	
Aug 16 1990	15.5	1.8	21.5
Aug 17 1990	18.5	3.6	
Aug 18 1990	17.0	0.2	
Aug 19 1990	24.5		
Aug 20 1990	27.5		
Aug 21 1990	27.0	0.4	
Aug 22 1990	22.0	2.4	19.0
Aug 23 1990	12.0	0.2	
Aug 24 1990	10.5		
Aug 25 1990	14.0	0.8	
Aug 26 1990	15.0	2.4	
Aug 27 1990	21.5		
Aug 28 1990	25.0		
Aug 29 1990	26.0	3.6	
Aug 30 1990	18.5	6.4	18.0
Aug 31 1990	15.5	6.8	

Continued . . .

Table A6 Concluded.

Date	Temperature °C high (Wetaskiwin)	Precipitation mm (Wetaskiwin)	Water Temp. °C (Coal Lake)
Sept 1 1990	21.5		
Sept 2 1990	20.0	1.0	
Sept 3 1990	19.0		
Sept 4 1990	24.0		
Sept 5 1990	25.0		
Sept 6 1990	24.0		18.5
Sept 7 1990	27.0		
Sept 8 1990	15.0	tr	
Sept 9 1990	22.0		
Sept 10 1990	25.5		17.0
Sept 11 1990	25.0		
Sept 12 1990	15.0	tr	
Sept 13 1990	22.0		16.0
Sept 14 1990	23.5		
Sept 15 1990	25.0		
Sept 16 1990	22.5	1.2	
Sept 17 1990	17.0		15.5
Sept 18 1990	22.5		
Sept 19 1990	18.0	0.4	
Sept 20 1990	17.0		14.5
Sept 21 1990	18.0		
Sept 22 1990	25.5		
Sept 23 1990	29.5		
Sept 24 1990	29.0		15.5
Sept 25 1990	25.0		
Sept 26 1990	16.5		
Sept 27 1990	14.0		14.0
Sept 28 1990	15.0		
Sept 29 1990	15.0		
Sept 30 1990	17.5		
Oct 1 1990	15.0		11.5
Oct 2 1990	12.5		
Oct 3 1990	10.0		
Oct 4 1990	1.0	20.6(snow)	9.0

Table A7 Coal Lake Cyanobacterial Genera Distribution

Date	Cyanobacterial Genera			LR/Biomass
	Aphanizomenon	*Microcystis*	*Gomphosphaeria*	(μg/g)
Aug 01 90	50	48	2	189
Aug 10 90	13	85	2	507
Aug 16 90	10	90	0	490
Aug 22 90	5	80	15	387
Aug 30 90	30	40	30	418
Sept 10 90	39	10	51	170
Sept 13 90	28	18	54	124
Sept 17 90	30	18	52	219
Sept 20 90	54	6	40	47
Sept 24 90	62	3	35	98
Oct 01 90	40	2	58	179
Oct 04 90	13	2	85	53
Oct 11 90	17	0	83	6

Table A8 Driedmeat Lake Cyanobacterial Genera Distribution

Date	Cyanobacterial Genera				LR/Biomass
	Aphanizomenon	*Microcystis*	*Gomphosphaeria*	*Anabaena*	(μg/g)
Aug 01 90	50	45	0	5	87
Aug 10 90					17
Aug 16 90	20	80	0	0	177
Sept 10 90	21	40	13	25	372
Sept 13 90	55	33	3	9	245
Sept 17 90	90.3	0.4	2.8	6.5	199

Table A9 Little Beaver Lake Cyanobacterial Genera Distribution

Date	Cyanobacterial Genera			LR/Biomass
	Aphanizomenon	*Microcystis*	Other	(μg/g)
Aug 01 90				8
Aug 10 90	95	5	0	24
Aug 16 90	95	5	0	12
Aug 22 90	95	5	0	34
Aug 30 90	90	10	0	53
Sept 10 90	83	17	0	20
Sept 13 90	94	6	0	27
Sept 20 90	95	5	0	209
Sept 24 90	95	5	0	435
Sept 27 90	95	3	2	74
Oct 01 90	91.7	0.4	7.9	30
Oct 04 90	100	0	0	25
Oct 11 90	100	0	0	4

Table A10 Coal Lake Data

Date	Water Temp. °C	Secchi Depth (m)	Total P (µg/L)	Total Dissolved P (µg/L)	Nitrate + Nitrite N (µg/L)	Ammonia N (µg/L)	Chlorophyll-a (µg/L)	%*Microcystis aeruginosa*	Mass LR/Biomass (µg/g)
1-Aug	22.0	1.10	119	47.2	20.8	8.3	49.5	48	189
10-Aug	22.0	2.10	125	92.5	5.2	50.9	16.7	85	507
16-Aug	21.5	1.45	195	140	13.7	376	42.9	90	490
22-Aug	19.0	1.60	246	173	23.8	224	20.2	80	387
30-Aug	18.0		206	126	48.2	8.2	29.7	40	418
6-Sep	18.5	1.40	165	75.0	2.6	162	41.4		
10-Sep	17.0	1.20	175	94.2	4.2		40.1	10	170
13-Sep	16.0	1.30					30.7	18	124
17-Sep	15.5	1.30	156	86.1	31.4	46.0	35.4	18	219
20-Sep	14.5	1.30					32.1	6	47
24-Sep	15.5	1.45	132	57.0	27.3	195	28.3	3	98
27-Sep	14.0	1.20					27.9	3	65
1-Oct	11.5	1.40	119	57.5	79.1	160	18.7	2	179
4-Oct	9.0	1.10					21.0	2	53
11-Oct			109	41.9	119.0	131	14.2	0	6

Table A11 Driedmeat Lake Data

Date	Water Temp. °C	Secchi Depth (m)	Total P (µg/L)	Total Dissolved P (µg/L)	Nitrate + Nitrite N (µg/L)	Ammonia N (µg/L)	Chlorophyll-a (µg/L)	%*Microcystis aeruginosa*	Mass LR/Biomass (µg/g)
1-Aug	22.0	1.20	226	174	35.7	146.4	11.0	45	87
10-Aug	24.0	0.90	148	95.9	4.2	5.6	21.9		17
16-Aug	21.5	2.55	348	256	13.8	172.9	1.81	80	177
22-Aug	18.0	1.80	255	208	42.9	478.0	8.9		
6-Sep	18.0	3.20	121	92.0	4.9	21.8	6.17		
10-Sep	17.0	2.55	192	129	21.1		9.75	40	372
13-Sep	16.0	2.20					13.0	33	245
17-Sep	15.0	1.50	164	121	57.9	36.2	11.3	0.4	199
20-Sep	13.5	1.90					13.9		
24-Sep	14.5	2.85	125	80.6	147.5	166.6	7.57		
27-Sep	13.5	2.00					15.8		
1-Oct	11.0	1.20	117	50.0	40.0	53.9	18.6		

Table A12 Little Beaver Lake Data

Date	Water Temp. °C	Secchi Depth (m)	Total P (µg/L)	Total Dissolved P (µg/L)	Nitrite + Nitrate N (µg/L)	Ammonia N (µg/L)	Chlorophyll-a (µg/L)	% *Microcystis aeruginosa*	Mass LR/Biomass (µg/g)
1-Aug	24.0	0.10	448	226	13.7	1	21.6		8
10-Aug	25.0	0.80	363	295	8.6	83.3	53.2	5	24
16-Aug	22.0	1.00	444	362	9.2	1262	101	5	12
22-Aug	20.0	0.80	517	414	6.9	2813	37.7	5	34
30-Aug	20.0	0.65	410	279	5.7	1302	61.7	10	53
6-Sep	18.0	1.40	335	282	110	808	8.46		
10-Sep	17.0	>1.90	427	394	116		7.27	17	20
13-Sep	16.0	>2.0					12.5	6	27
17-Sep	14.5	1.50	455	418	37	2296	11.1		
20-Sep	13.5	1.50					20.2	5	209
24-Sep	14.0	>1.80	445	358	114	2563	31.0	5	435
27-Sep	13.0	>2.20					10.5	3	74
1-Oct	10.0	1.20	406	287	338	2355	34.0	0.4	30
4-Oct	7.0	1.80					11.9	0	25
11-Oct			274	216	325	2278	24.9	0	4

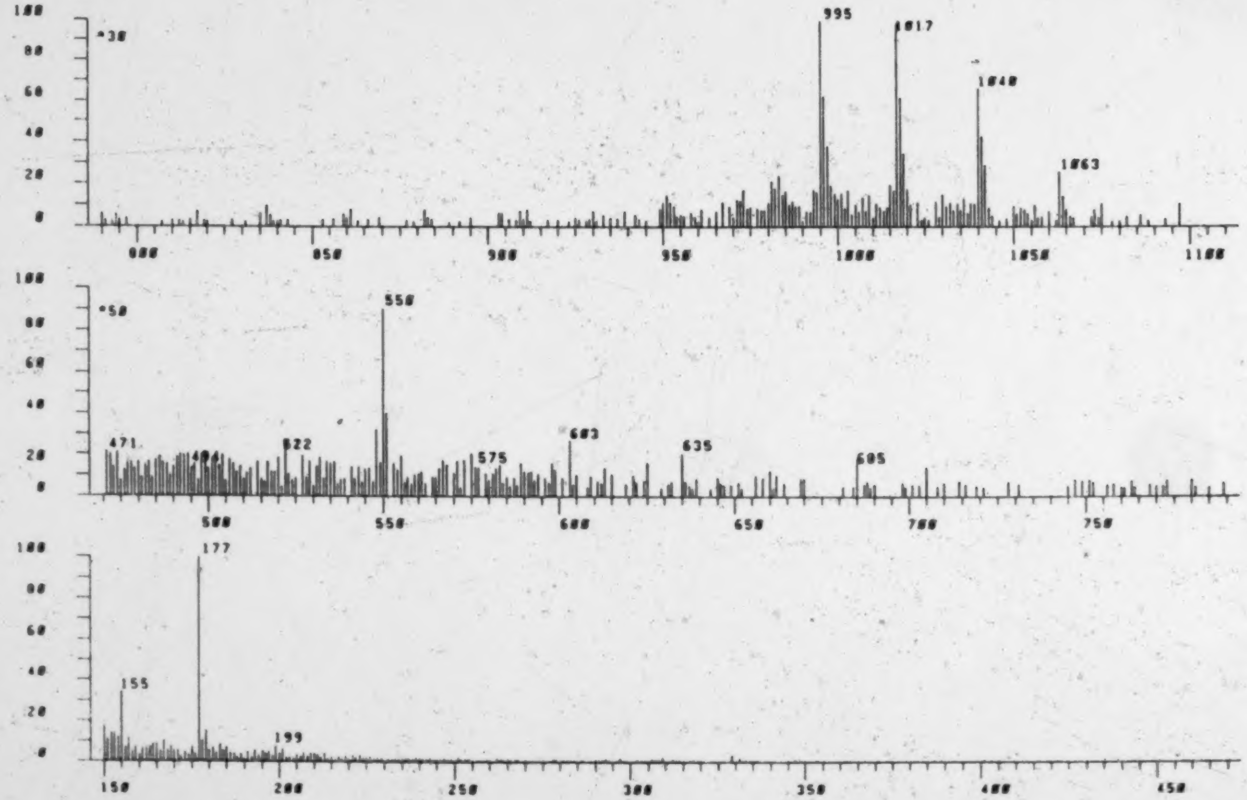

Figure A1 Positive Ion FAB Spectra of Isolated Microcystin LR in Magic Bullet Matrix

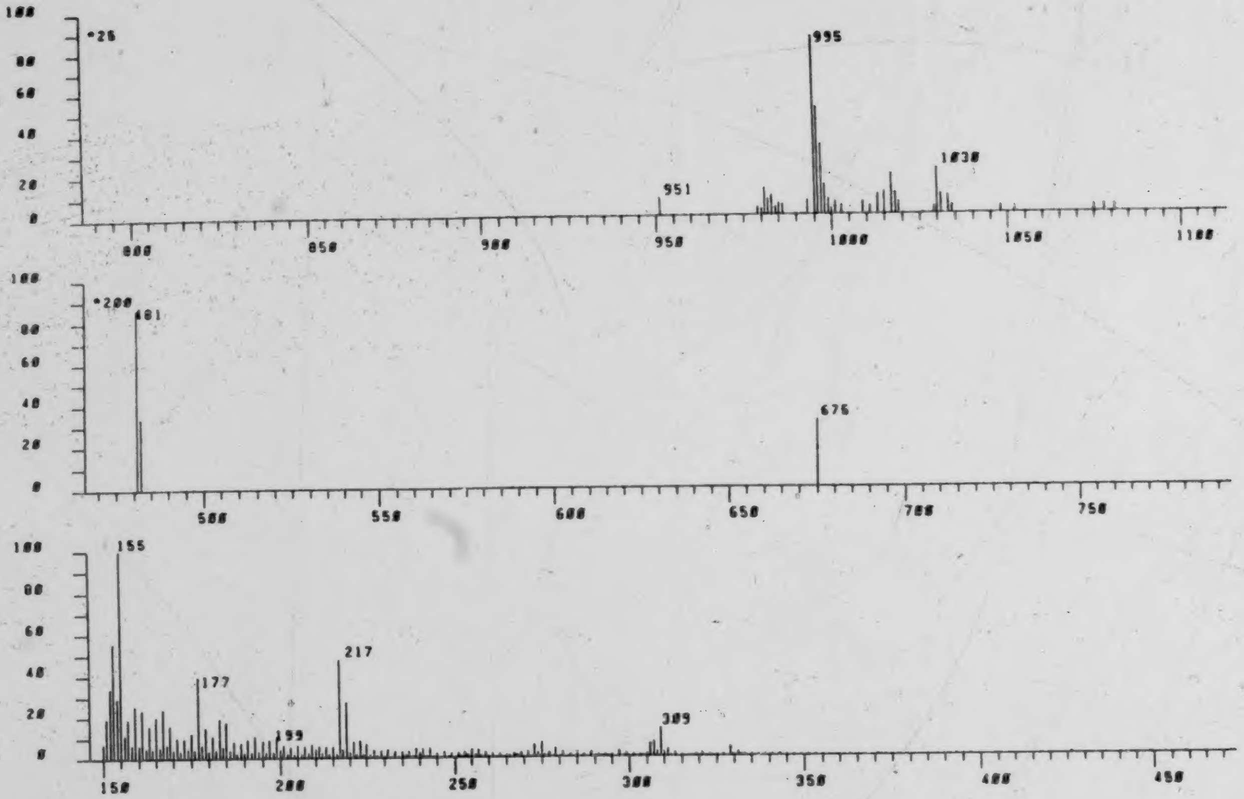

Figure A2 Positive Ion FAB Spectra of Microcystin LR Standard in Magic Bullet Matrix